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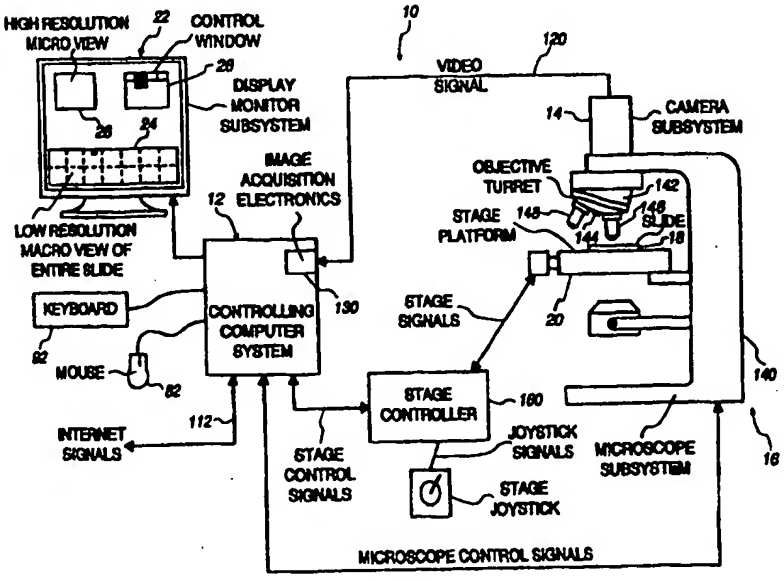


(57) Abstract

A computer-controlled microscope (16) captures a plurality of images at a low magnification. These images are tiled to create a reconstructed image (24) of an entire specimen. The user selects one or more areas of the reconstructed image. The computer-controlled microscope (16) then captures a plurality of high resolution images (26) of the selected areas for display to the user.

METHOD AND APPARATUS FOR ACQUIRING AND RECONSTRUCTING MAGNIFIED SPECIMEN IMAGES FROM A COMPUTER-CONTROLLED MICROSCOPE

Field of the Invention

This invention relates to a method of and apparatus for acquiring and recording digital images of an optical image viewed through a computer-controlled automated microscope and also, to using the latter in a quantitative analysis of plant or biological specimens.

Background of the Invention

In the image analysis and quantification of DNA from tissue sections as disclosed in United States Patent 4,741,031, and also especially in the immunohisto-chemistry assays on the kinds of cell analysis systems disclosed in United States Patents 5,086,476; 5,202,931; and 5,252,487 issued to Bacus, there is a problem of first locating the cancer regions for analysis under low power and then remembering them when performing the analysis under higher power. There is a need, a requirement to image and digitally record an object in a relatively flat plane at high resolution/magnification. Today, it is impractical to construct an optical image sensor large enough to cover the entire image area e.g., of a specimen on a microscope slide, at the required resolution. This is because lens size and resolution/magnification issues limit the size of the field of view of magnified objects and their resulting images. Viewing through a microscope is akin to viewing through a periscope in that one sees a very small field of view even at low magnifications, such as 1.25X. A pathologist using a microscope often scans a slide to obtain in his mind an overall view or sense of what constitutes the specimen and he remembers the general locations of the diagnostically significant, small pieces of the specimen. Usually, these are the diseased areas, such as malignant or potentially malignant portions of

important diagnostic regions. However, as set forth in my co-pending patent application Serial No. 701,974, filed August 23, 1996, if these regions are located, important very sensitive diagnostic measurements can be performed, which patent application is incorporated by reference as if fully reproduced herein. For example, as disclosed in the aforesaid patent application, assays are made of a variety of tissue types, both human and animal for analysis of neoplasia in tissue, for pre-invasive cancer in tissue, and the effects on the tissue of chemopreventive agents. A quantitative analysis by image processing techniques is performed on tissue types, having various architectural features, such as breast tissue, colon tissue, prostate tissue, esophageal tissue, skin tissue, cervix tissue, etc. These tissues have different morphologies, and they undergo different neoplasias usually resulting from a cellular mutation, as may be enhanced by a carcinogen, or resulting from a cellular proliferation rate enhanced by hormones, growth factors, or other inducers of abnormal tissue growth. Often it is desired to quantify small changes in the neoplasia when it is incipient or through a series of analyses performed at close time intervals to measure whether the neoplasia progression is increasing or has been slowed, stopped or regressed.

Usually, the tissue specimens are cut to expose the basal layer for review under the microscope. Typically, the quantitative measurements are performed at 40x to obtain 100 to 400 tissue images. The 40x objective provides a narrow field of view of a very small portion of the entire basal layer. Often, the basal layer is somewhat elongated and generally linear such as a basal layer in a rat esophagus; and the analysis of the basal layer requires examining it along its length. The basal layer in a mouse colon is more in the form of an irregular, circular shape; and the analysis of this basal layer requires traveling about this circular shape. In

images from the same location on the specimen. Heretofore, the practice of pathology has been relatively limited to the use of microscopes and to the pathologist having to use the microscope to review the particular specimen.

There is a need for a dynamic system whereby one or more or several pathologists, including a consulting pathologist, may view the same area simultaneously and interact with one another either in diagnosis or in analysis. Also, it would be best if the images from the specimen could be stored so that a pathologist could easily examine the images at his leisure using an intranet or Internet browser at a later date merely by accessing the particular web site where the images are located.

A similar problem exists on the Internet or intranet where a pathologist may receive a single field of view magnified image taken from a specimen over the Internet or the intranet on his browser. The pathologist must be provided with explanations to coordinate the high resolution view with the lower resolution view. The number of views available to the pathologist is very limited, and the pathologist is unable to select other views or to scroll to neighboring views at the areas that are most interesting to the pathologist.

There are available on the market computer-controlled, automated microscopes such as those sold by Carl Zeiss, Inc., Thornwood, N.J., under the name Axioplan 2 for taking photographic images of a specimen in the microscopic field of view. Those particular microscopes have computer-controlled and automatically adjusted subsystems, such as an illumination subsystem, a focusing subsystem, a diaphragm or optical stops subsystem, an objective lens subsystem, or a filtering subsystem. As an operator selects changes from one objective lens, such as one providing low magnification, e.g., 4x, to a higher magnification, e.g., 40x, the

interest on the specimen. Sometimes, a technician will do a first assay and analysis. A pathologist will return to the selected points of interest or other points of interest for review and analysis. One concern with respect to a quantitative analysis of breast cancer tissue or prostate biopsy tissue samples to pap smears or other tests for various cancers or the like is that a particularly suspicious point in the tissue may be overlooked and missed during the visual assay or for selection for an automated review analysis. When observing at high magnifications, the field of view is limited to very small area of the specimen. Hence, the observer has difficulty in knowing and remembering the actual, exact location of this small periscopic view within the very large whole specimen.

Often, also the problem is finding or locating the tissue or cells for view at high magnification so that artifacts and/or blank spaces on the slide are not viewed. A number of approaches have been proposed to prescreen and locate by an X and Y address the cells or small points of interest from a very large number of potential points of interest.

There are currently available commercial services for prescreening pap smears where one can mail in slides and the service will do a microscopic prescan at high magnification for suspected or suspicious areas of interest which are marked and given address locations, and also a video tape of the slide specimen is returned by this service to the sender. The sender then reviews the areas of interest located during the prescreening and/or the video tape to complete the analysis.

In an attempt to locate and allow review of specified points of interest, U.S. Patent No. 5,428,690 to Bacus discloses a system for prescreening of a field of cells on a specimen slide at low magnification before the viewer. When seeing a point of interest to be viewed at high magnification, the viewer will operate a switch

the specimen, the pathologist's field of view of the actual specimen is only the small circle of view that is coming through the microscope objective lens. This does not help the pathologist locate suspicious areas of interest as in a prescreening of the entire tissue. The pathologist may switch to the lowest magnification to get the largest field of view of a small section of the specimen, but he never views the entire specimen at any magnification. Also, there is no image analysis quantitative testing from the received images at the diagnostic center; and no quantitative assaying is done with these images at the diagnostic center.

There is a particular interest today in using the Internet system because it is so readily accessible by users at a low cost and using a computer and viewing screen connected to the computer. One problem with trying to do any transmission of digitized, microscopic, highly magnified images over the Internet is that the bandwidth is too narrow to accommodate the tremendous amount of stored data which needs to be transmitted. There is a need for a system which would allow a pathologist or another person, to be able to perform tissue analysis or quantitative assays using a standard computer terminal from a location remote from the automated microscope system that digitized the optical images.

Summary of the Invention

In accordance with the present invention, a person such as a pathologist at a computer terminal may view on a computer screen or monitor digitized images of a microscope specimen at different magnifications as selected by the person. Further, the person may receive on the screen a low magnification, reconstructed image of the entire specimen to aid the person in interactively selecting points of interest on the specimen, such as along a basal layer of a tissue specimen.

reproduced, spatially adjacent high resolution, digitized images of the selected area of interest. More specifically, the specimen, when it was first scanned at low magnification to provide a macro view of the specimen, the addresses or locations of the tile images and/or pixels for the composite image were acquired. Therefore, any selected region of interest in the macro image has locations to which the microscopic stage may be automatically repositioned under a changed, higher magnification lens to acquire higher magnification, digitized image tiles that can be assembled into a micro image. Herein, both the macro and micro images are formed of adjacent digitized image tiles which have been coordinated to reproduce spatially the original image that was scanned.

It is the high magnification images, usually at 40x, that were analyzed using image processing techniques as disclosed in the aforesaid patent application, to provide an assay or numerical histological data for the specimen.

In accordance with the preferred embodiment of the invention, the pathologist may desire to see a larger region for analysis at high resolution than can be accommodated at this magnification on his high magnification viewing screen. The pathologist can quickly change the view on his screen to view adjacent, highly magnified, digitized image tiles at this high resolution by scrolling up or down or right to left to shift these digitized, adjacent image tiles into view on the screen. Thus, even at a higher magnification of a region the pathologist is able to obtain a much larger view than the small field for the objective lens in use of adjacent tissue or cells to give him a broader, overall perspective of what is happening or what has happened in specific section of a specimen. For instance, a pathologist may want to see at high magnification and to assay at this high magnification, a

Likewise, the reconstructed, high magnification images can be transmitted over such narrow band width channels.

The preferred microscope is fully computer controlled, a pathologist or other person having a split screen computer such as a PC, can be connected to the microscope and operate it from a remote location with the computer microscope system to digitize and construct the macro image. Likewise, the pathologist can navigate to points of interest and with the computer microscope system to digitize and construct the desired micro images. With the present invention, there is no need for a specialized microscope at each remote location nor for a broad band channel to send video signals in real time between the diagnostic center and the remote location.

Brief Description of the Drawings

FIG. 1 is a screen view of a system embodying the present invention showing a low magnification image of a specimen on a microscope slide in one window, a high magnification image of a portion of the low magnification image selected by a region marker and a control window;

FIG. 2 is a view of a display screen of the apparatus embodying the present invention showing the control window a low magnification window having a plurality of high magnification micro image regions delineated therein and a high magnification window including one or more of the micro image regions;

FIG. 3 is a view similar to FIG. 2 including the control window but also including a low magnification region from the slide showing regions marked by a histology grade or structure through automatic analysis of tissue and a high magnification window showing markings related to the grading or histology grade yielded by the automatic analysis of tissue in combination with a window showing a numerical score;

FIG. 4 is a block diagram of the apparatus embodying the present invention;

FIG. 15A is a view of a reconstructed macro image of a mouse colon;

FIG. 16 is a schematic view of an analysis from regions of a basal layer;

FIG. 16A is a schematic view of an analysis to provide to a Z score; and

FIG. 17 is a schematic view showing texture analysis tests for regions.

Detailed Description of the Preferred Embodiment

Referring now to the drawings, and especially to Figs. 4 and 5, apparatus for synthesizing low magnification and high magnification microscopic images is shown therein and generally identified by reference numeral 10. The system includes a computer 12 which is a dual Pentium Pro personal computer in combination with a Hitachi HV-C20 video camera 14 associated with a Zeiss Axioplan 2 microscope 16. The computer system 12 is able to receive signals from the camera 14 which captures light from the microscope 16 having a microscope slide 18 positioned on an LUDL encoded motorized stage 20. The encoded motorized stage 20 includes a MAC 2000 stage controller for controlling the stage in response to the computer 12. A microscope slide 18 includes a biological specimen 21 which is to be viewed by the microscope and whose image is to be digitized both at low magnification and at high magnification as selected by a user. The low magnification digitized image is then displayed on a 21 inch Iiyama video display monitor 22 having resolution of 1600 by 1200 to provide display screens of the type shown in Figs. 1 through 3 including a low magnification image 24, for instance, at 1.25 power, a high magnification image 26, for instance at 40 power and a control window or image 28. The low magnification image may have identified therein a region 30 which is reproduced at high magnification in high magnification screen or window 26 so that a pathologist or other operator of the system

coupled to the system bus 40. A network interface, such as a network interface card 104, is connected to the system bus and can provide signals via a channel 106 to other portions of a network or internet to which the system may be connected. Likewise, signals can be sent out of the system through a modem 110 connected to the ISA bus 52 and may be sent via a channel 112, for instance, to the internet. A printer 116 is connected via a parallel I/O controller 118 to the system bus in order to provide printouts as appropriate of screens and other information as it is generated. A serial I/O controller 122 is connected to the system bus and has connected to it a camera controller 124 which is coupled to CCD sensors 126 in the cameras. The CCD sensors 126 supply pixel or image signals representative of what is found on the slide 18 to an Epix pixci image acquisition controller 130 coupled to the PCI bus 50.

The microscope 16 includes a base 140 having a stage 20 positioned thereon as well as an objective turret 142 having a plurality of objectives 144, 146 and 148 thereon. The objective 144, for instance, may be of 1.25x objective. The objective 146 may be a 20X objective. The objective 148 may be a 40X objective. Signals from the camera sensors and controller are supplied over a bus 128 to the image acquisition system where they are digitized and supplied to the PCI bus for storage in RAM or for backing storage on the hard disk 62.

When a specimen is on the slide 18 the stage 20 may be manipulated under the control of the computer through a stage controller 160 coupled to the serial I/O controller 122. Likewise, a microscope controller 162 controls aspects of the microscope such as the illumination, the color temperature or spectral output of a lamp 168 and the like. For instance, in normal operation, when a specimen is placed on the slide, specimen slide 18 is placed on the stage 20 in a step

step 209d. In a step 209e, a command may be received to scan or acquire the higher magnification image for display in screen 26. The image may then be archived for later analysis, displayed or analyzed immediately.

In order to perform the magnification called for in step 208, the overall illumination and control of the microscope will be controlled so that in a step 210 the objective turret 142 will be rotated to place the higher power objective above the slide 18. In a step 212 voltage to the lamp will be changed to adjust the lamp 168 to provide the proper illumination and color temperature as predetermined for the selected objective. In a step 214, the condenser diaphragm 176 will have its opening selected as appropriate to provide the proper illumination for that objective. In a step 216, the filter turret 180 will select the proper light wavelength filter to be supplied to the camera sensors. For instance, a red, blue or green filter, as appropriate, particularly if the specimen has been stained. In a step 218 the field diaphragm 174 will have its opening changed. In a step 220 the neutral density filter wheel 170 will select a neutral density filter and in a step 222 the neutral density filter wheel 172 will also select a neutral density filter. In a step 224 the X, Y and Z offsets will be used for reconstruction of the recorded image at the magnification and in a step 226 the current position will be read from encoders in the stage which are accurate to .10 micron.

In order to identify the selected region the mouse is moved to that area of the region in a pointing operation in a step 240 as shown in FIG. 9. The mouse may be moved to draw a box around the region selected. In a step 242 the X and Y screen points are computed for the edges of the regions selected and the computed image or pixel points are translated to stage coordinate points in order to control the stage of the microscope. In a step 244 a list of all of the X fields for positioning

of a reference point 290 at its upper left hand corner. Since the size of the macro image tile 288 is known, the next macro image tile 292 may be placed contiguous with it by moving the stage appropriately and by measuring the location of the stage from the stage in counters without the necessity of performing any image manipulation. The image tiles 288 and 292 may be abutted without any substantial overlap or they may be overlapped slightly, such as a one pixel with overlap, which is negligible insofar as blurring of any adjacent edges of abutted image tiles. The upper left hand corner 300 of the tile 292 defines the rest of 292 and other tiles can be so defined. Micro image tiles can likewise be defined so that they are contiguous but not substantially overlapping, as would interfere with the composite image. This avoids the problems encountered with having to perform extended computations on digital images in a frame storer or multiple frame storage in order to match or bring the images into contiguity without blurriness at the edges of contiguous image tiles. It may be appreciated as shown in FIG. 2 that the low power image 24 has a plurality of micro images defined therein which are tiled and which are shown in higher magnification as individual tiles 312, 314, 316 and the like in FIG. 2. In addition, the region 310 when magnified as shown in the window 26 may exceed the bounds of the window and thus the window may include scroll bars or other means for allowing the image 310 which is larger than the window 26 to be examined from within the window 26.

The stage 200 is best seen in FIG. 11A and includes the X and Y stepper motors 279 and 281 with their respective encoders, which provide a closed loop system to give the .1 micron accuracy versus the usual 5 or 6 micron accuracy of most microscope stages without a closed loop system. This closed loop system and this very high accuracy allow the abutting of the tile images for both high magnification and low magnification images

An additional feature of the system includes a plurality of networked workstations coupled to a first computer console 12 having a display screen 22 connected to the microscope 14. Satellite work stations 350 and 352 are substantially identical to the work station 12 including respective computers 354 and 356 coupled to displays 358 and 360. The devices can be manipulated through input devices 360 and 362 which may include a keyboard, mouse and the like. Also a third device can be connected including a work station 370, having a display 372, a computer 374 and an input device 376. Each of the devices is connected over respective network lines 380, 382, 384 to the computer 12 which transmission may be via either net or the like. Each of the different operators at the physically separate viewing stations can locate regions from the view of entire tissue cross sections via a macro view and label the regions for subsequent scanning and/or quantitative analysis. A single operator at the instrument station 12 can locate regions to view the entire tissue cross section. Those regions can be labeled for subsequent scanning and/or quantitative analysis with subsequent review and physically remote viewing stations, for instance, in an operating room or in individual pathologists' signout areas in order to review analysis results while still maintaining and reviewing the entire macro view of the tissue and/or the individual stored images from which the quantitative results were obtained. The viewing stations 350, 352 and 370 can comprise desk top computers, laptops, etc. There is no need for a microscope at the network stations 350, 352 and 370.

In a still further alternative embodiment, remote workstations 400, 402, 404, 406 and 408 may be connected through a server 410 which may be supplied via a packet switched network. The server 410 and may be a hypertext transport protocol based server of the type used for the World Wide Web or may be a telnet type

be appreciated that numerous changes and modifications will occur to those skilled in the art, and it is intended in the appended claims to cover all those changes and modifications which followed in the true spirit and scope of the present invention.

digitized microscopic images from a specimen on a microscope support, said method comprising the steps of:

scanning and digitizing a specimen at a low magnification through a microscope to acquire digitized images;

displaying to the observer a low magnification, digitized image of the specimen;

selecting a segment of the specimen from the low magnification digitized image for viewing at a higher magnification greater than the low magnification;

scanning the selected segment at the higher magnification and digitizing the image of the segment to acquire digitized images; and

storing the respective, low magnification, digitized images and the high magnification, digitized images of the segment.

6. The method of Claim 5 wherein the step of scanning and digitizing at low magnification further includes the steps of:

positioning and coordinating spatially a plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and

providing a macro image of the specimens having a field of view substantially larger than the field of view yielded by the objective lens used for the low magnification scanning.

7. A method in accordance with Claim 6 including the step of reducing the size of the composite, macro image before displaying the composite macro image of the specimen to the viewer.

8. A method in accordance with Claim 6 wherein the step of selecting and digitizing the low magnification, digitized image further includes the step

and simultaneously displaying the high magnification image of the segment at a position located by the marker on the low magnification image.

13. The method of Claim 5 including the step of shifting the marker to different segments and viewing at high magnification each of the selected segments; and marking on the low magnification image, each of the segments viewed at high magnification.

14. The method of Claim 5 including the steps of locating a computer-controlled microscope system used to make and store the digitized images on the Internet having a web site therefor; including the step of connecting an observer's computer and viewing screen over the Internet to the computer-controlled microscope at the web site in real time and transmitting the digitized signals for low magnification image and high magnification image from the web site to the observer's computer and viewing screen.

15. The method of Claim 5 including the step of enhancing the reconstructed, high magnification image by color filtering.

16. The method of Claim 5 including the step of providing a full color, low magnification, digitized image to the observer for aiding the observer in selecting a plurality of areas of interest on a priority basis.

17. The method of Claim 5 including the steps of:

analyzing the specimen on the slide with image analysis tests; and

22. The method of Claim 21 wherein the step of scanning and digitizing at low magnification further includes the steps of:

positioning and coordinating spatially a plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and

providing a macro image of the specimens having a field of view substantially larger than the field of view yielded by the objective lens used for the low magnification scanning.

23. A method of using digitized, microscope images of a specimen having images at a first resolution at a low magnification and images at a second resolution at a higher magnification, the method comprising the steps of:

showing to a user a series of digitized, tiled and stored images abutted to form a macro image from the specimen at a first resolution;

selecting from the stored macro image an area thereon to the viewer to be viewed at higher resolution;

showing to the user a series of abutted, digitized, tiled, stored images at the higher magnification and higher resolution to the viewer to be viewed at higher resolution.

24. A method in accordance with Claim 23 including the further step of shifting by user, back and forth between lower and higher resolution, stored images to analyze the specimen.

25. A method in accordance with Claim 24 including the further step of marking on the stored macro image the area to be viewed at higher resolution.

28. A method in accordance with Claim 27 including the step of marking on the macro image, the location of the micro image, so that the observer understands where the micro image is located on the macro image.

29. A method in accordance with Claim 27 including the step of selecting several segments each for viewing as a micro image and marking the location of each micro image on the composite image.

30. A method in accordance with Claim 29 including the step of recording the macro image with the marks thereon to provide a record of the segments viewed by the observer at high magnification to provide an audit trail for a later auditing of the segments viewed at higher resolution.

31. A method of acquiring and analyzing biological specimens with a computer-controlled microscope; said method comprising the steps of:

providing a biological specimen in position for scanning through the computer-controlled microscope;

scanning and digitizing the biological specimen through the microscope at a first, low magnification and acquiring and storing a digitized image of the specimen in color;

displaying the stored, low magnification, digitized image in color to an observer to provide a macro image for viewing by the observer;

selecting a segment of the colored, digitized, low magnification image for viewing at a higher magnification than the low magnification;

scanning the segment at the higher magnification and acquiring a colored digitized image of the segment at the higher magnification to provide a micro image of the segment;

an imaging subsystem connected to a microscope to acquire and digitize the images of the specimen;

a first optical system and image acquisition and image storing system having a first objective lens to acquire and arrange a stored series of images at a low magnification to provide a macro digitized image of the specimen having a field of view larger than the field of view from the objective lens used for the low magnification acquisition of images;

a second optical and image acquisition and image storing system having a second, higher magnification objective lens to acquire and arrange a stored series of images at higher magnification and to provide a micro view image of the segment having a field of view larger than the field of view from the higher magnification objective lens.

35. A system in accordance with Claim 34 wherein the first image subsystem includes an X and Y position storage device for storing the X and Y coordinates for the macro image.

36. A system in accordance with Claim 34 wherein the second image acquisition subsystem has a subsystem for reconstructing high magnification image tiles with their X and Y coordinates positioned to reproduce spatially high resolution image tiles into the micro image as if the micro image was in the original image.

37. A system in accordance with Claim 34 wherein the first image acquisition subsystem has a subsystem for reconstructing low resolution image tiles with their X and Y coordinates positioned to reproduce spatially the low resolution tiles into a macro image of the specimen.

40. A method for analyzing biological specimens by an image analysis system having a computer-controlled, automated microscope comprising the following steps:

placing a biological specimen in a microscope for viewing;

using a computer terminal to control the image analysis system and microscope to acquire low magnification, image tiles of the specimen and to provide low magnification, composite, macro image from the plurality of image tiles;

displaying the macro image on a screen of the computer terminal;

interactively selecting at least one point of interest on the displayed macro, specimen image for viewing at a higher magnification;

sending signals from the computer terminal to control the image analysis system and the computer-controlled microscope to acquire a plurality of higher magnification, image tiles and to provide a low magnification, composite micro image; and

analyzing the micro images of the specimen.

41. A storage medium having digitized images of a specimen from a microscope slide comprising:

a storage medium;

a first series of contiguous, multiple digitized images at a first magnification abutted against each other to create an overall low resolution view of several adjacent, original microscope images assembled together;

the first series of multiple, digitized images taken from at least a portion of a specimen on the slide;

a second series of contiguous, multiple, digitized images at a second higher magnification abutted against each other to create a high resolution view of several adjacent, original microscope images assembled together, taken from said portion of the specimen; and

Fig. 1

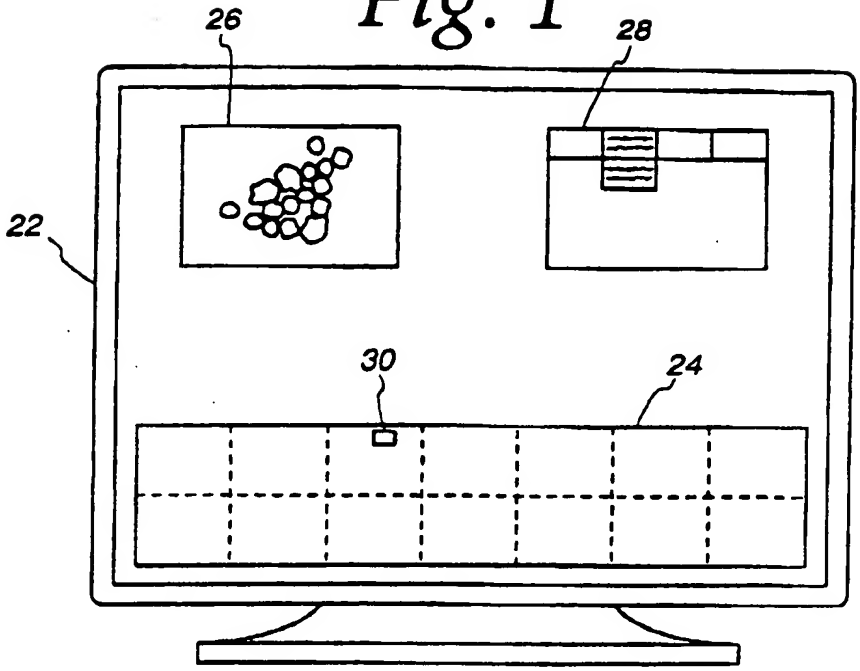


Fig. 2

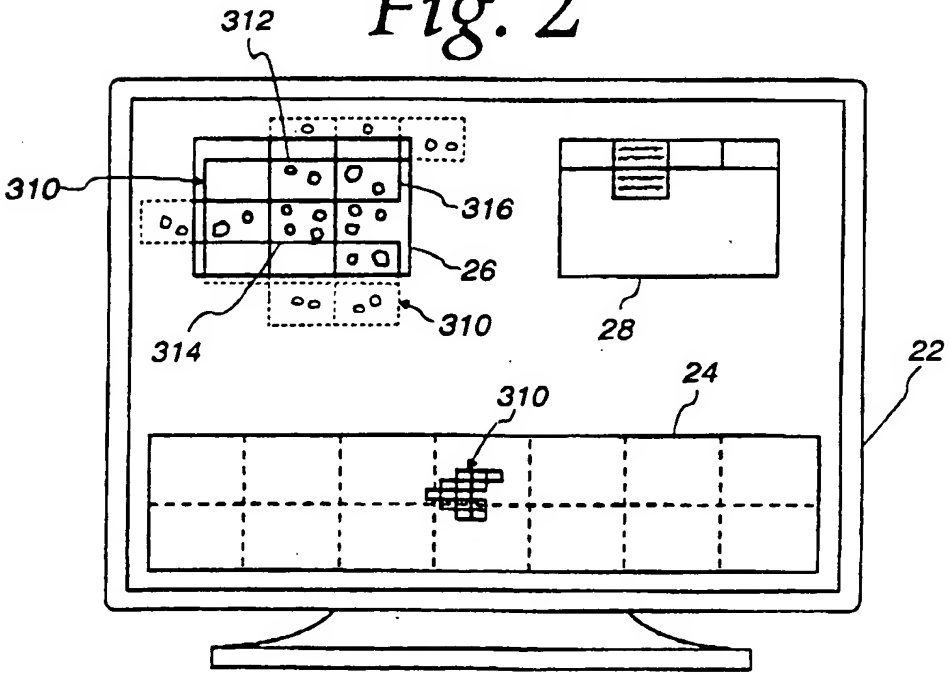
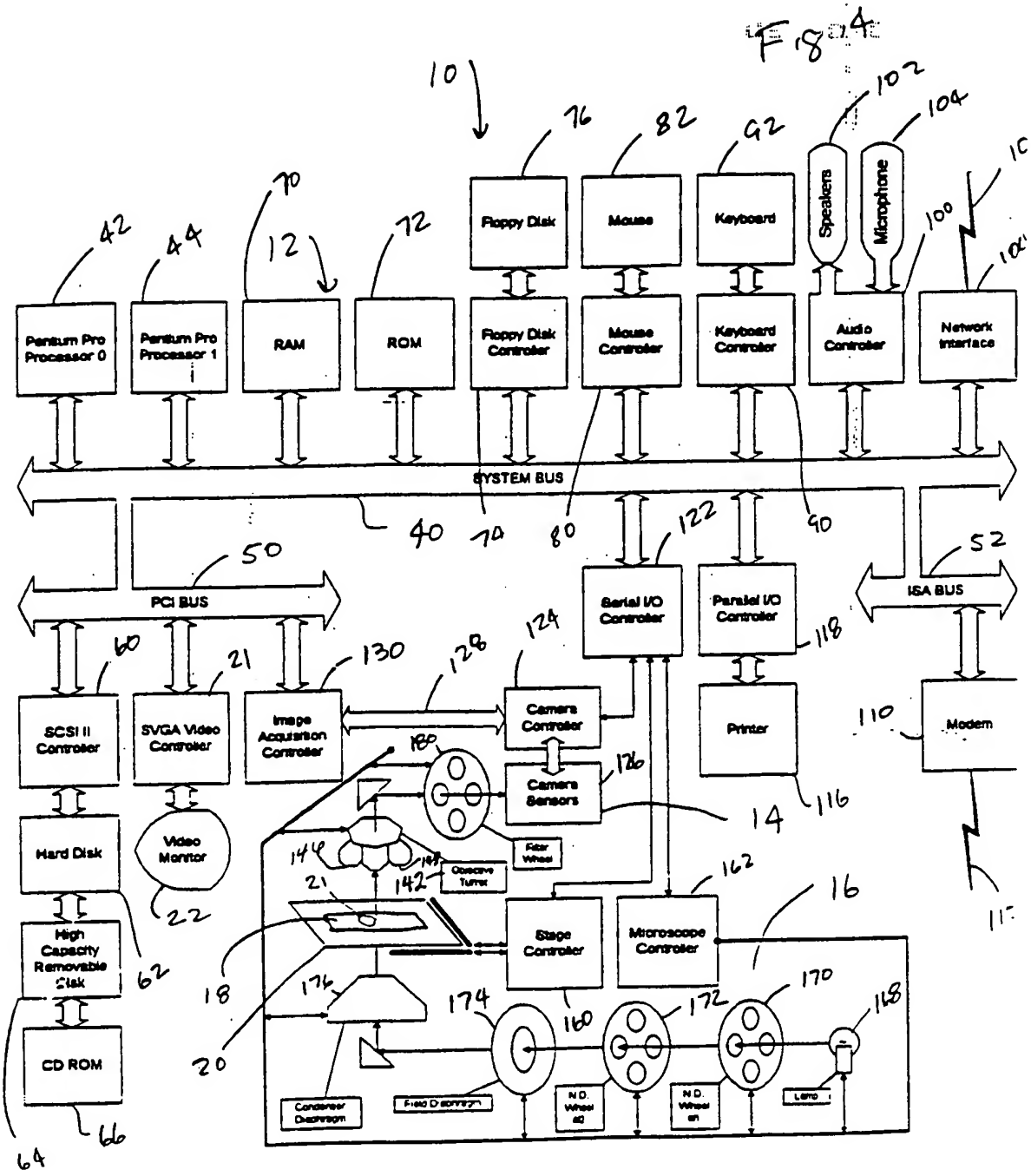


Fig 4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76
82
92
102
104
Floppy Disk
Mouse
Keyboard
Speakers
Microphone
42
44
70
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72
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Pentium Pro Processor 1
RAM
ROM
Floppy Disk Controller
Mouse Controller
Keyboard Controller
Audio Controller
Network Interface
SYSTEM BUS
40
74
80
90
122
50
52
PCI BUS
Serial I/O Controller
Parallel I/O Controller
ISA BUS
60
21
130
128
124
118
SCSI II Controller
SVGA Video Controller
Image Acquisition Controller
Camera Controller
Printer
110
Modem
180
126
Camera Sensors
14
116
Hard Disk
Video Monitor
Filter Wheel
146
142
Objective Turret
162
16
21
Stage Controller
Microscope Controller
High Capacity Removable Disk
62
18
176
174
160
172
170
168
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CD ROM
Condenser Diaphragm
Field Diaphragm
N.D. Wheel #2
N.D. Wheel #1
Lamp
66
64

Fig. 6

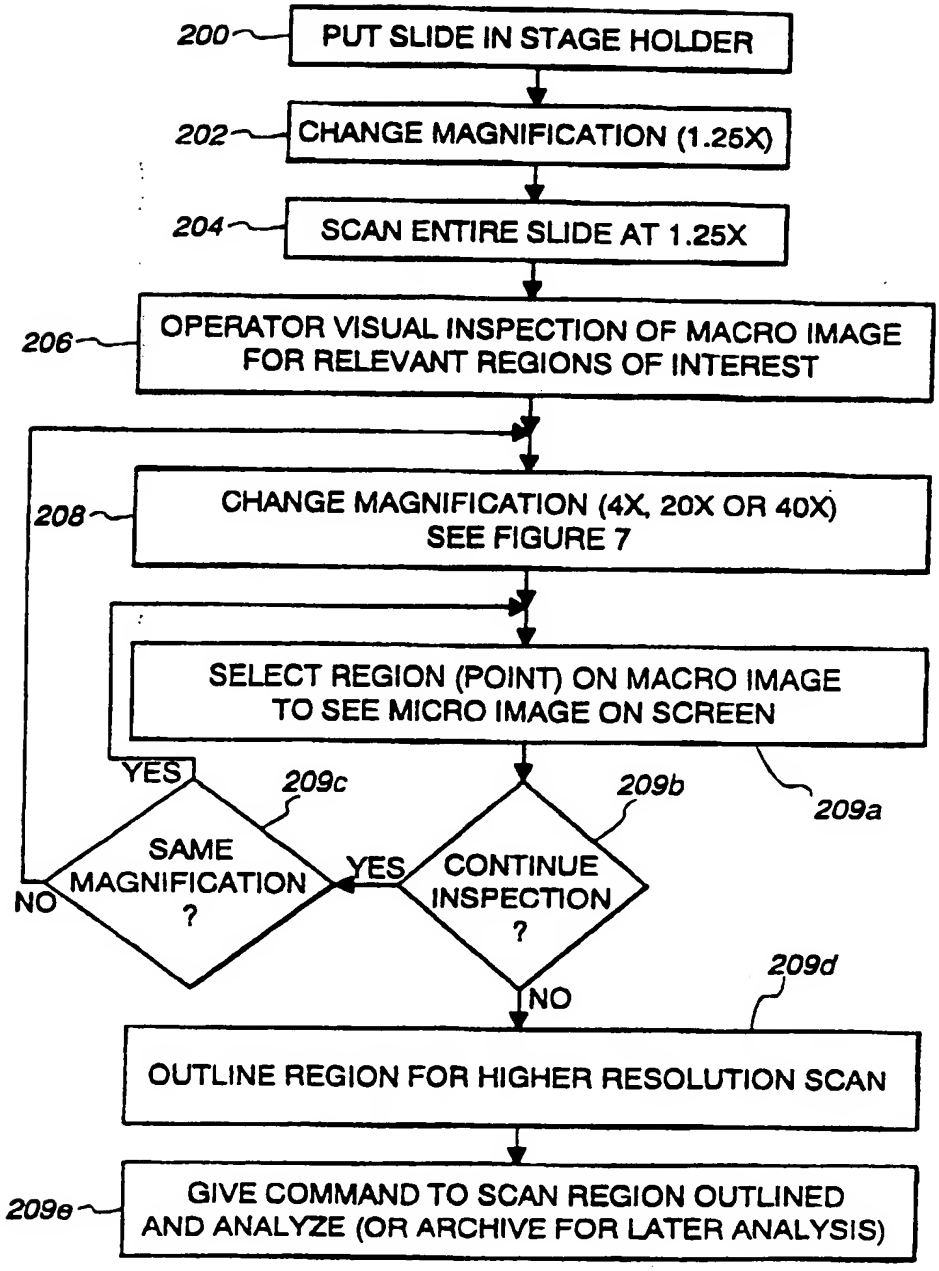


Fig. 8

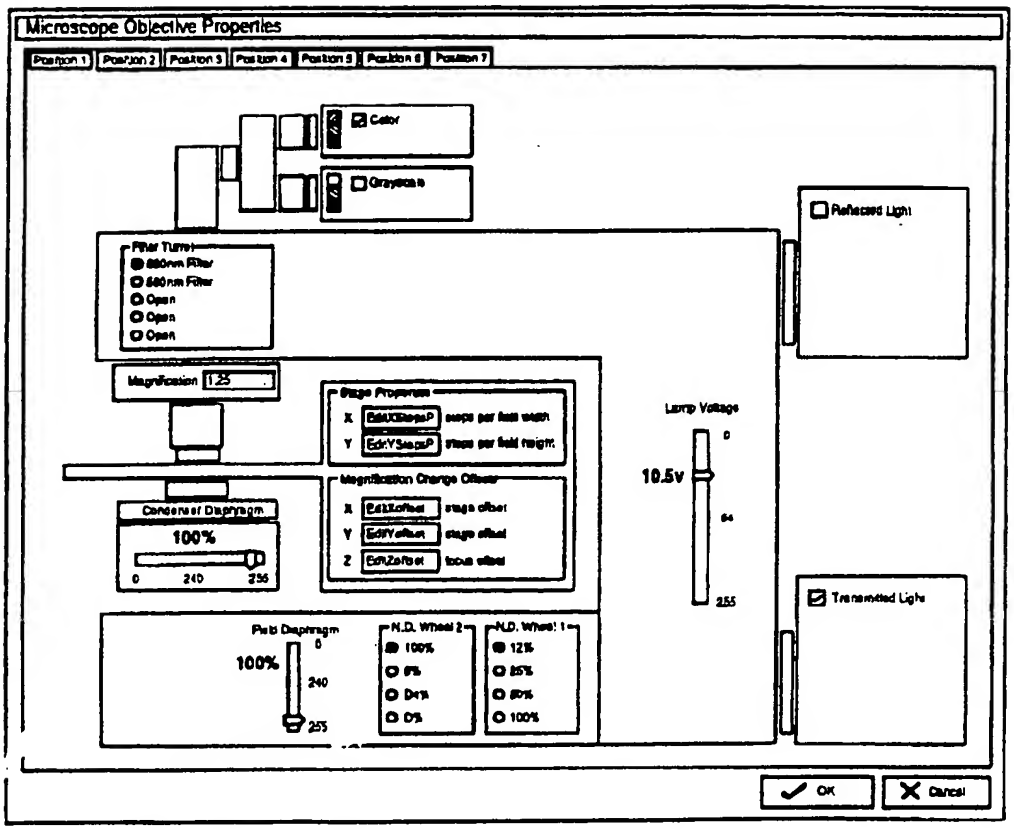

Microscope Objective Properties
Position 1
Position 2
Position 3
Position 4
Position 5
Position 6
Position 7
Color
Grayscale
Reflected Light
Filter Turret
Open
Open
Open
Magnification
125
Stage Properties
X
Y
Magnification Change Offsets
X
Y
Z
Condenser Diaphragm
100%
0
240
255
Lamp Voltage
10.5v
0
64
255
Transmitted Light
Field Diaphragm
100%
0
240
255
N.D. Wheel 2
100%
N.D. Wheel 1
12%
OK
Cancel

Fig. 11

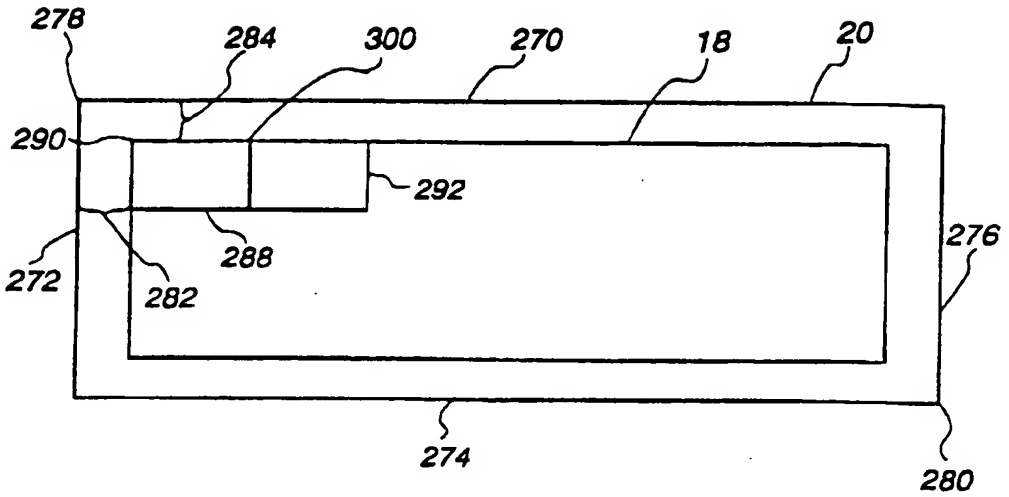
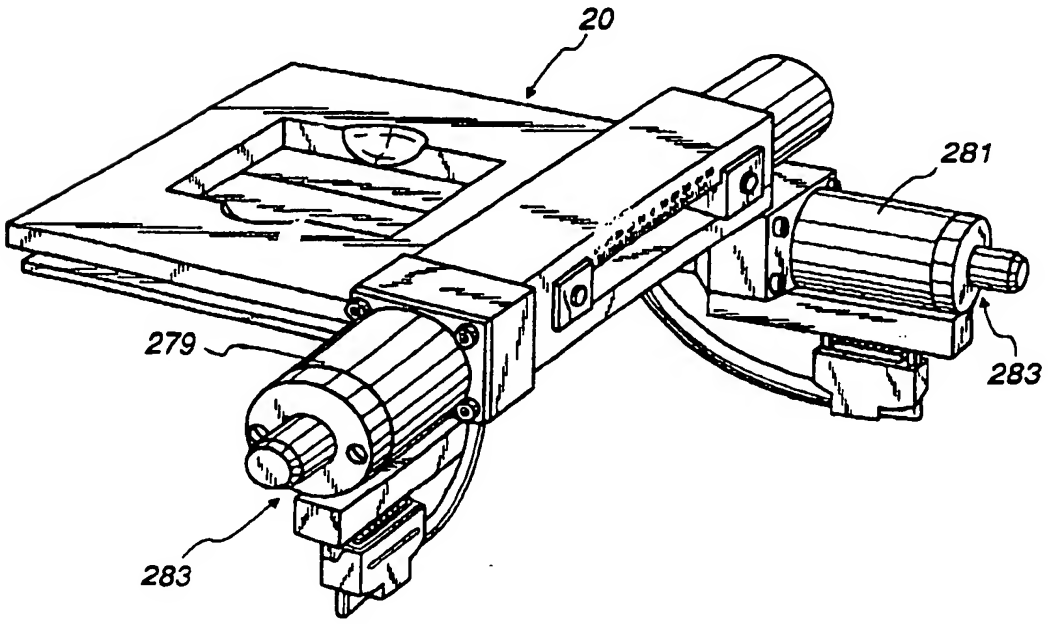


Fig. 11A



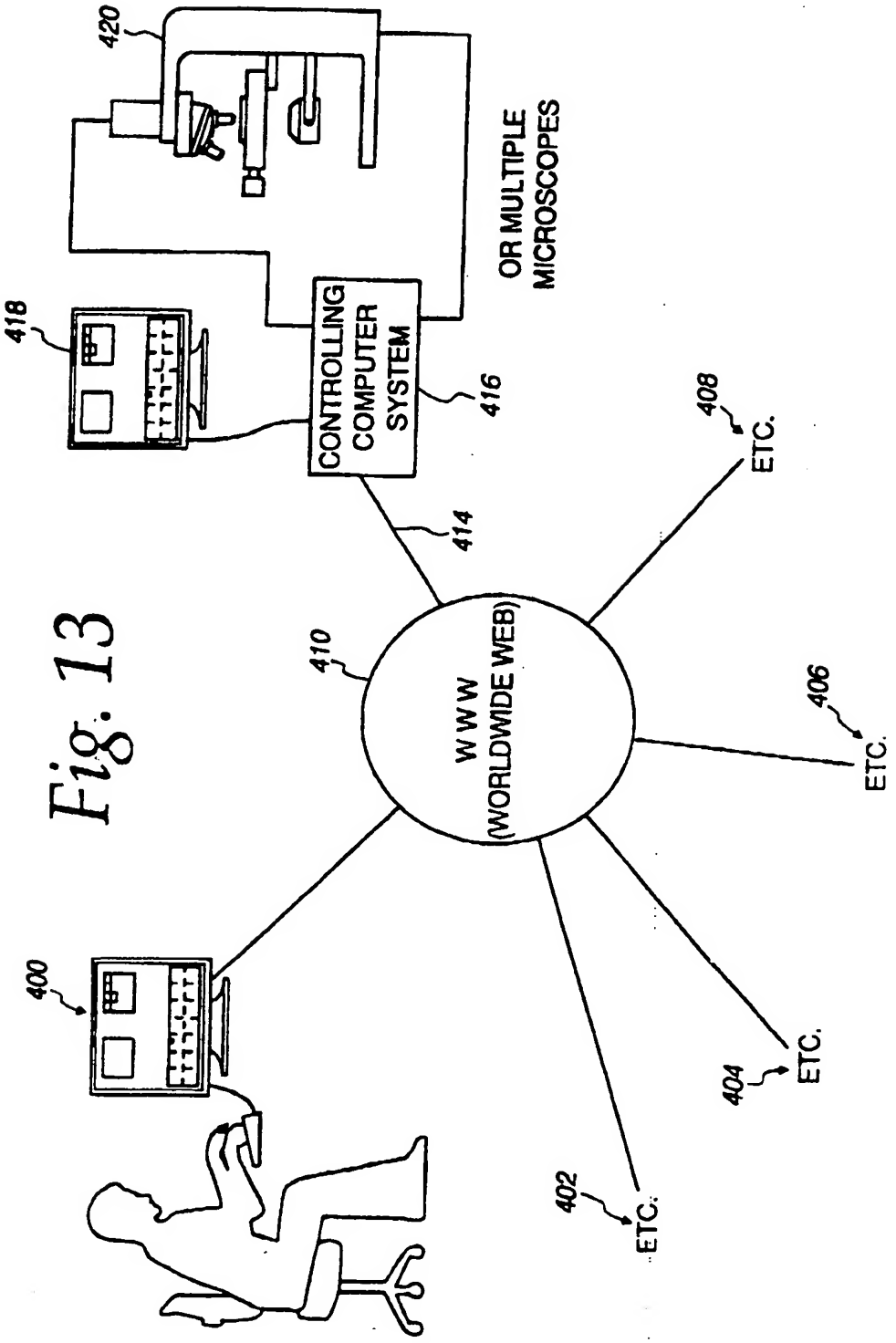

420
OR MULTIPLE MICROSCOPES
418
CONTROLLING COMPUTER SYSTEM
416
408
ETC.
414
410
W W W (WORLDWIDE WEB)
406
ETC.
400
404
ETC.
402
ETC.

Fig. 13

Tissue Section Image Acquisition
(Mouse Colon)

FIG 200
Fig. 15A

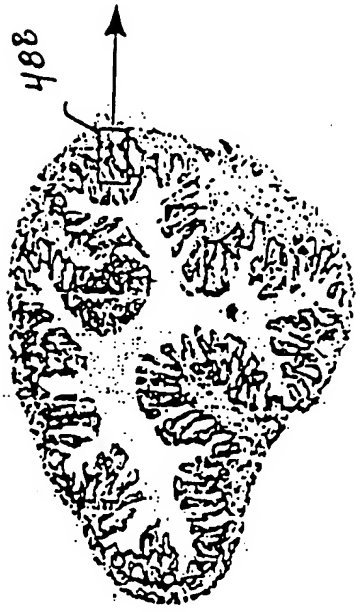


FIG 200
15

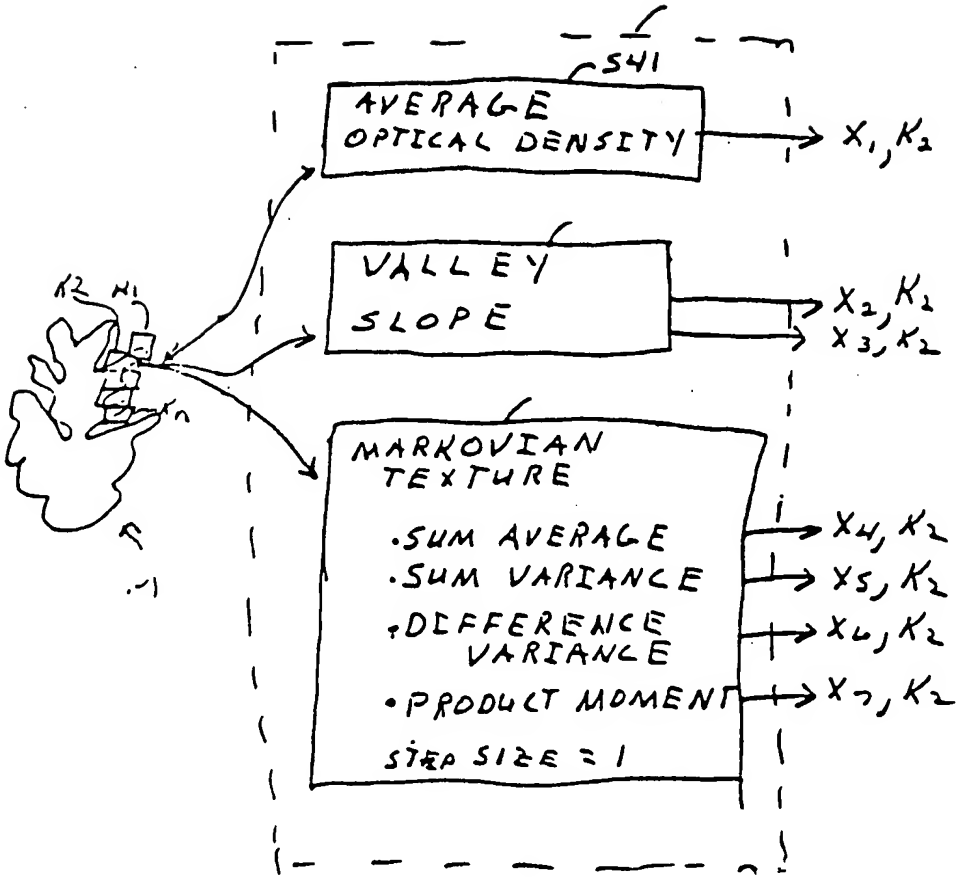

541
AVERAGE OPTICAL DENSITY
X_1, K_2
VALLEY SLOPE
X_2, K_2
X_3, K_2
MARKOVIAN TEXTURE
•SUM AVERAGE
•SUM VARIANCE
•DIFFERENCE VARIANCE
• PRODUCT MOMENT
STEP SIZE = 1
X_4, K_2
X_5, K_2
X_6, K_2
X_7, K_2
K2 H1
Xn

FIG-~~23~~ 17

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International Bureau

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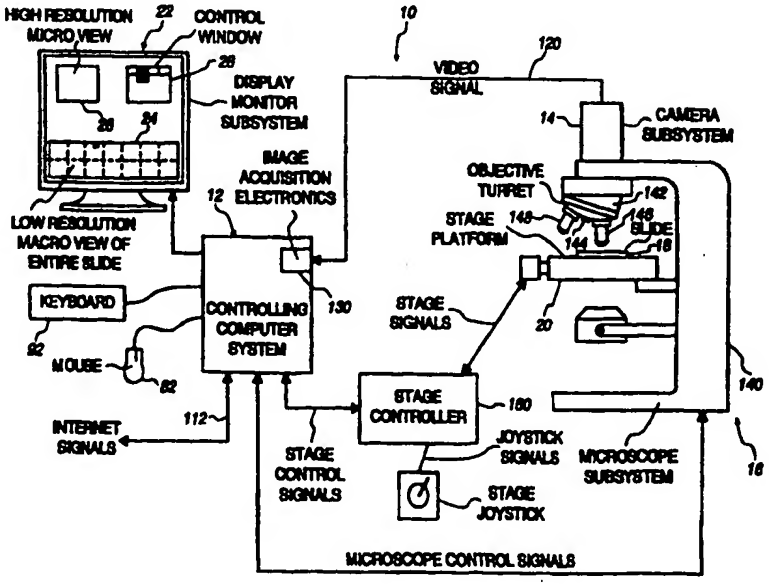
(54) Title: METHOD AND APPARATUS FOR ACQUIRING AND RECONSTRUCTING MAGNIFIED SPECIMEN IMAGES FROM A COMPUTER-CONTROLLED MICROSCOPE



(57) Abstract

A computer-controlled microscope (16) captures a plurality of images at a low magnification. These images are tiled to create a reconstructed image (24) of an entire specimen. The user selects one or more areas of the reconstructed image. The computer-controlled microscope (16) then captures a plurality of high resolution images (26) of the selected areas for display to the user.

*(Referred to in PCT Gazette No. 49/1998, Section II)

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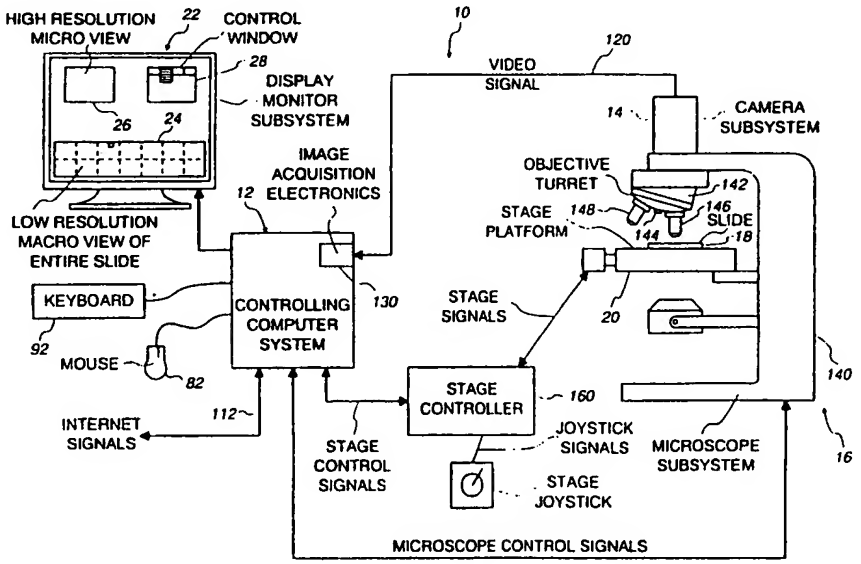


(57) Abstract

A computer-controlled microscope (16) captures a plurality of images at a low magnification. These images are tiled to create a reconstructed image (24) of an entire specimen. The user selects one or more areas of the reconstructed image. The computer-controlled microscope (16) then captures a plurality of high resolution images (26) of the selected areas for display to the user.

METHOD AND APPARATUS FOR ACQUIRING AND RECONSTRUCTING MAGNIFIED SPECIMEN IMAGES FROM A COMPUTER-CONTROLLED MICROSCOPE

Field of the Invention

This invention relates to a method of and apparatus for acquiring and recording digital images of an optical image viewed through a computer-controlled automated microscope and also, to using the latter in a quantitative analysis of plant or biological specimens.

Background of the Invention

In the image analysis and quantification of DNA from tissue sections as disclosed in United States Patent 4,741,031, and also especially in the immunohisto-chemistry assays on the kinds of cell analysis systems disclosed in United States Patents 5,086,476; 5,202,931; and 5,252,487 issued to Bacus, there is a problem of first locating the cancer regions for analysis under low power and then remembering them when performing the analysis under higher power. There is a need, a requirement to image and digitally record an object in a relatively flat plane at high resolution/magnification. Today, it is impractical to construct an optical image sensor large enough to cover the entire image area e.g., of a specimen on a microscope slide, at the required resolution. This is because lens size and resolution/magnification issues limit the size of the field of view of magnified objects and their resulting images. Viewing through a microscope is akin to viewing through a periscope in that one sees a very small field of view even at low magnifications, such as 1.25X. A pathologist using a microscope often scans a slide to obtain in his mind an overall view or sense of what constitutes the specimen and he remembers the general locations of the diagnostically significant, small pieces of the specimen. Usually, these are the diseased areas, such as malignant or potentially malignant portions of

important diagnostic regions. However, as set forth in my co-pending patent application Serial No. 701,974, filed August 23, 1996, if these regions are located, important very sensitive diagnostic measurements can be performed, which patent application is incorporated by reference as if fully reproduced herein. For example, as disclosed in the aforesaid patent application, assays are made of a variety of tissue types, both human and animal for analysis of neoplasia in tissue, for pre-invasive cancer in tissue, and the effects on the tissue of chemopreventive agents. A quantitative analysis by image processing techniques is performed on tissue types, having various architectural features, such as breast tissue, colon tissue, prostate tissue, esophageal tissue, skin tissue, cervix tissue, etc. These tissues have different morphologies, and they undergo different neoplasias usually resulting from a cellular mutation, as may be enhanced by a carcinogen, or resulting from a cellular proliferation rate enhanced by hormones, growth factors, or other inducers of abnormal tissue growth. Often it is desired to quantify small changes in the neoplasia when it is incipient or through a series of analyses performed at close time intervals to measure whether the neoplasia progression is increasing or has been slowed, stopped or regressed.

Usually, the tissue specimens are cut to expose the basal layer for review under the microscope. Typically, the quantitative measurements are performed at 40x to obtain 100 to 400 tissue images. The 40x objective provides a narrow field of view of a very small portion of the entire basal layer. Often, the basal layer is somewhat elongated and generally linear such as a basal layer in a rat esophagus; and the analysis of the basal layer requires examining it along its length. The basal layer in a mouse colon is more in the form of an irregular, circular shape; and the analysis of this basal layer requires traveling about this circular shape. In

images from the same location on the specimen. Heretofore, the practice of pathology has been relatively limited to the use of microscopes and to the pathologist having to use the microscope to review the particular specimen.

There is a need for a dynamic system whereby one or more or several pathologists, including a consulting pathologist, may view the same area simultaneously and interact with one another either in diagnosis or in analysis. Also, it would be best if the images from the specimen could be stored so that a pathologist could easily examine the images at his leisure using an intranet or Internet browser at a later date merely by accessing the particular web site where the images are located.

A similar problem exists on the Internet or intranet where a pathologist may receive a single field of view magnified image taken from a specimen over the Internet or the intranet on his browser. The pathologist must be provided with explanations to coordinate the high resolution view with the lower resolution view. The number of views available to the pathologist is very limited, and the pathologist is unable to select other views or to scroll to neighboring views at the areas that are most interesting to the pathologist.

There are available on the market computer-controlled, automated microscopes such as those sold by Carl Zeiss, Inc., Thornwood, N.J., under the name Axioplan 2 for taking photographic images of a specimen in the microscopic field of view. Those particular microscopes have computer-controlled and automatically adjusted subsystems, such as an illumination subsystem, a focusing subsystem, a diaphragm or optical stops subsystem, an objective lens subsystem, or a filtering subsystem. As an operator selects changes from one objective lens, such as one providing low magnification, e.g., 4x, to a higher magnification, e.g., 40x, the

interest on the specimen. Sometimes, a technician will do a first assay and analysis. A pathologist will return to the selected points of interest or other points of interest for review and analysis. One concern with respect to a quantitative analysis of breast cancer tissue or prostate biopsy tissue samples to pap smears or other tests for various cancers or the like is that a particularly suspicious point in the tissue may be overlooked and missed during the visual assay or for selection for an automated review analysis. When observing at high magnifications, the field of view is limited to very small area of the specimen. Hence, the observer has difficulty in knowing and remembering the actual, exact location of this small periscopic view within the very large whole specimen.

Often, also the problem is finding or locating the tissue or cells for view at high magnification so that artifacts and/or blank spaces on the slide are not viewed. A number of approaches have been proposed to prescreen and locate by an X and Y address the cells or small points of interest from a very large number of potential points of interest.

There are currently available commercial services for prescreening pap smears where one can mail in slides and the service will do a microscopic prescan at high magnification for suspected or suspicious areas of interest which are marked and given address locations, and also a video tape of the slide specimen is returned by this service to the sender. The sender then reviews the areas of interest located during the prescreening and/or the video tape to complete the analysis.

In an attempt to locate and allow review of specified points of interest, U.S. Patent No. 5,428,690 to Bacus discloses a system for prescreening of a field of cells on a specimen slide at low magnification before the viewer. When seeing a point of interest to be viewed at high magnification, the viewer will operate a switch

the specimen, the pathologist's field of view of the actual specimen is only the small circle of view that is coming through the microscope objective lens. This does not help the pathologist locate suspicious areas of interest as in a prescreening of the entire tissue. The pathologist may switch to the lowest magnification to get the largest field of view of a small section of the specimen, but he never views the entire specimen at any magnification. Also, there is no image analysis quantitative testing from the received images at the diagnostic center; and no quantitative assaying is done with these images at the diagnostic center.

There is a particular interest today in using the Internet system because it is so readily accessible by users at a low cost and using a computer and viewing screen connected to the computer. One problem with trying to do any transmission of digitized, microscopic, highly magnified images over the Internet is that the bandwidth is too narrow to accommodate the tremendous amount of stored data which needs to be transmitted. There is a need for a system which would allow a pathologist or another person, to be able to perform tissue analysis or quantitative assays using a standard computer terminal from a location remote from the automated microscope system that digitized the optical images.

Summary of the Invention

In accordance with the present invention, a person such as a pathologist at a computer terminal may view on a computer screen or monitor digitized images of a microscope specimen at different magnifications as selected by the person. Further, the person may receive on the screen a low magnification, reconstructed image of the entire specimen to aid the person in interactively selecting points of interest on the specimen, such as along a basal layer of a tissue specimen.

reproduced, spatially adjacent high resolution, digitized images of the selected area of interest. More specifically, the specimen, when it was first scanned at low magnification to provide a macro view of the specimen, the addresses or locations of the tile images and/or pixels for the composite image were acquired. Therefore, any selected region of interest in the macro image has locations to which the microscopic stage may be automatically repositioned under a changed, higher magnification lens to acquire higher magnification, digitized image tiles that can be assembled into a micro image. Herein, both the macro and micro images are formed of adjacent digitized image tiles which have been coordinated to reproduce spatially the original image that was scanned.

It is the high magnification images, usually at 40x, that were analyzed using image processing techniques as disclosed in the aforesaid patent application, to provide an assay or numerical histological data for the specimen.

In accordance with the preferred embodiment of the invention, the pathologist may desire to see a larger region for analysis at high resolution than can be accommodated at this magnification on his high magnification viewing screen. The pathologist can quickly change the view on his screen to view adjacent, highly magnified, digitized image tiles at this high resolution by scrolling up or down or right to left to shift these digitized, adjacent image tiles into view on the screen. Thus, even at a higher magnification of a region the pathologist is able to obtain a much larger view than the small field for the objective lens in use of adjacent tissue or cells to give him a broader, overall perspective of what is happening or what has happened in specific section of a specimen. For instance, a pathologist may want to see at high magnification and to assay at this high magnification, a

Likewise, the reconstructed, high magnification images can be transmitted over such narrow band width channels.

The preferred microscope is fully computer controlled, a pathologist or other person having a split screen computer such as a PC, can be connected to the microscope and operate it from a remote location with the computer microscope system to digitize and construct the macro image. Likewise, the pathologist can navigate to points of interest and with the computer microscope system to digitize and construct the desired micro images. With the present invention, there is no need for a specialized microscope at each remote location nor for a broad band channel to send video signals in real time between the diagnostic center and the remote location.

Brief Description of the Drawings

FIG. 1 is a screen view of a system embodying the present invention showing a low magnification image of a specimen on a microscope slide in one window, a high magnification image of a portion of the low magnification image selected by a region marker and a control window;

FIG. 2 is a view of a display screen of the apparatus embodying the present invention showing the control window a low magnification window having a plurality of high magnification micro image regions delineated therein and a high magnification window including one or more of the micro image regions;

FIG. 3 is a view similar to FIG. 2 including the control window but also including a low magnification region from the slide showing regions marked by a histology grade or structure through automatic analysis of tissue and a high magnification window showing markings related to the grading or histology grade yielded by the automatic analysis of tissue in combination with a window showing a numerical score;

FIG. 4 is a block diagram of the apparatus embodying the present invention;

FIG. 15A is a view of a reconstructed macro image of a mouse colon;

FIG. 16 is a schematic view of an analysis from regions of a basal layer;

FIG. 16A is a schematic view of an analysis to provide to a Z score; and

FIG. 17 is a schematic view showing texture analysis tests for regions.

Detailed Description of the Preferred Embodiment

Referring now to the drawings, and especially to Figs. 4 and 5, apparatus for synthesizing low magnification and high magnification microscopic images is shown therein and generally identified by reference numeral 10. The system includes a computer 12 which is a dual Pentium Pro personal computer in combination with a Hitachi HV-C20 video camera 14 associated with a Zeiss Axioplan 2 microscope 16. The computer system 12 is able to receive signals from the camera 14 which captures light from the microscope 16 having a microscope slide 18 positioned on an LUDL encoded motorized stage 20. The encoded motorized stage 20 includes a MAC 2000 stage controller for controlling the stage in response to the computer 12. A microscope slide 18 includes a biological specimen 21 which is to be viewed by the microscope and whose image is to be digitized both at low magnification and at high magnification as selected by a user. The low magnification digitized image is then displayed on a 21 inch Iiyama video display monitor 22 having resolution of 1600 by 1200 to provide display screens of the type shown in Figs. 1 through 3 including a low magnification image 24, for instance, at 1.25 power, a high magnification image 26, for instance at 40 power and a control window or image 28. The low magnification image may have identified therein a region 30 which is reproduced at high magnification in high magnification screen or window 26 so that a pathologist or other operator of the system

coupled to the system bus 40. A network interface, such as a network interface card 104, is connected to the system bus and can provide signals via a channel 106 to other portions of a network or internet to which the system may be connected. Likewise, signals can be sent out of the system through a modem 110 connected to the ISA bus 52 and may be sent via a channel 112, for instance, to the internet. A printer 116 is connected via a parallel I/O controller 118 to the system bus in order to provide printouts as appropriate of screens and other information as it is generated. A serial I/O controller 122 is connected to the system bus and has connected to it a camera controller 124 which is coupled to CCD sensors 126 in the cameras. The CCD sensors 126 supply pixel or image signals representative of what is found on the slide 18 to an Epix pixci image acquisition controller 130 coupled to the PCI bus 50.

The microscope 16 includes a base 140 having a stage 20 positioned thereon as well as an objective turret 142 having a plurality of objectives 144, 146 and 148 thereon. The objective 144, for instance, may be of 1.25x objective. The objective 146 may be a 20X objective. The objective 148 may be a 40X objective. Signals from the camera sensors and controller are supplied over a bus 128 to the image acquisition system where they are digitized and supplied to the PCI bus for storage in RAM or for backing storage on the hard disk 62.

When a specimen is on the slide 18 the stage 20 may be manipulated under the control of the computer through a stage controller 160 coupled to the serial I/O controller 122. Likewise, a microscope controller 162 controls aspects of the microscope such as the illumination, the color temperature or spectral output of a lamp 168 and the like. For instance, in normal operation, when a specimen is placed on the slide, specimen slide 18 is placed on the stage 20 in a step

step 209d. In a step 209e, a command may be received to scan or acquire the higher magnification image for display in screen 26. The image may then be archived for later analysis, displayed or analyzed immediately.

In order to perform the magnification called for in step 208, the overall illumination and control of the microscope will be controlled so that in a step 210 the objective turret 142 will be rotated to place the higher power objective above the slide 18. In a step 212 voltage to the lamp will be changed to adjust the lamp 168 to provide the proper illumination and color temperature as predetermined for the selected objective. In a step 214, the condenser diaphragm 176 will have its opening selected as appropriate to provide the proper illumination for that objective. In a step 216, the filter turret 180 will select the proper light wavelength filter to be supplied to the camera sensors. For instance, a red, blue or green filter, as appropriate, particularly if the specimen has been stained. In a step 218 the field diaphragm 174 will have its opening changed. In a step 220 the neutral density filter wheel 170 will select a neutral density filter and in a step 222 the neutral density filter wheel 172 will also select a neutral density filter. In a step 224 the X, Y and Z offsets will be used for reconstruction of the recorded image at the magnification and in a step 226 the current position will be read from encoders in the stage which are accurate to .10 micron.

In order to identify the selected region the mouse is moved to that area of the region in a pointing operation in a step 240 as shown in FIG. 9. The mouse may be moved to draw a box around the region selected. In a step 242 the X and Y screen points are computed for the edges of the regions selected and the computed image or pixel points are translated to stage coordinate points in order to control the stage of the microscope. In a step 244 a list of all of the X fields for positioning

of a reference point 290 at its upper left hand corner. Since the size of the macro image tile 288 is known, the next macro image tile 292 may be placed contiguous with it by moving the stage appropriately and by measuring the location of the stage from the stage in counters without the necessity of performing any image manipulation. The image tiles 288 and 292 may be abutted without any substantial overlap or they may be overlapped slightly, such as a one pixel with overlap, which is negligible insofar as blurring of any adjacent edges of abutted image tiles. The upper left hand corner 300 of the tile 292 defines the rest of 292 and other tiles can be so defined. Micro image tiles can likewise be defined so that they are contiguous but not substantially overlapping, as would interfere with the composite image. This avoids the problems encountered with having to perform extended computations on digital images in a frame storer or multiple frame storage in order to match or bring the images into contiguity without blurriness at the edges of contiguous image tiles. It may be appreciated as shown in FIG. 2 that the low power image 24 has a plurality of micro images defined therein which are tiled and which are shown in higher magnification as individual tiles 312, 314, 316 and the like in FIG. 2. In addition, the region 310 when magnified as shown in the window 26 may exceed the bounds of the window and thus the window may include scroll bars or other means for allowing the image 310 which is larger than the window 26 to be examined from within the window 26.

The stage 200 is best seen in FIG. 11A and includes the X and Y stepper motors 279 and 281 with their respective encoders, which provide a closed loop system to give the .1 micron accuracy versus the usual 5 or 6 micron accuracy of most microscope stages without a closed loop system. This closed loop system and this very high accuracy allow the abutting of the tile images for both high magnification and low magnification images

An additional feature of the system includes a plurality of networked workstations coupled to a first computer console 12 having a display screen 22 connected to the microscope 14. Satellite work stations 350 and 352 are substantially identical to the work station 12 including respective computers 354 and 356 coupled to displays 358 and 360. The devices can be manipulated through input devices 360 and 362 which may include a keyboard, mouse and the like. Also a third device can be connected including a work station 370, having a display 372, a computer 374 and an input device 376. Each of the devices is connected over respective network lines 380, 382, 384 to the computer 12 which transmission may be via either net or the like. Each of the different operators at the physically separate viewing stations can locate regions from the view of entire tissue cross sections via a macro view and label the regions for subsequent scanning and/or quantitative analysis. A single operator at the instrument station 12 can locate regions to view the entire tissue cross section. Those regions can be labeled for subsequent scanning and/or quantitative analysis with subsequent review and physically remote viewing stations, for instance, in an operating room or in individual pathologists' signout areas in order to review analysis results while still maintaining and reviewing the entire macro view of the tissue and/or the individual stored images from which the quantitative results were obtained. The viewing stations 350, 352 and 370 can comprise desk top computers, laptops, etc. There is no need for a microscope at the network stations 350, 352 and 370.

In a still further alternative embodiment, remote workstations 400, 402, 404, 406 and 408 may be connected through a server 410 which may be supplied via a packet switched network. The server 410 and may be a hypertext transport protocol based server of the type used for the World Wide Web or may be a telnet type

be appreciated that numerous changes and modifications will occur to those skilled in the art, and it is intended in the appended claims to cover all those changes and modifications which followed in the true spirit and scope of the present invention.

digitized microscopic images from a specimen on a microscope support, said method comprising the steps of:

scanning and digitizing a specimen at a low magnification through a microscope to acquire digitized images;

displaying to the observer a low magnification, digitized image of the specimen;

selecting a segment of the specimen from the low magnification digitized image for viewing at a higher magnification greater than the low magnification;

scanning the selected segment at the higher magnification and digitizing the image of the segment to acquire digitized images; and

storing the respective, low magnification, digitized images and the high magnification, digitized images of the segment.

6. The method of Claim 5 wherein the step of scanning and digitizing at low magnification further includes the steps of:

positioning and coordinating spatially a plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and

providing a macro image of the specimens having a field of view substantially larger than the field of view yielded by the objective lens used for the low magnification scanning.

7. A method in accordance with Claim 6 including the step of reducing the size of the composite, macro image before displaying the composite macro image of the specimen to the viewer.

8. A method in accordance with Claim 6 wherein the step of selecting and digitizing the low magnification, digitized image further includes the step

and simultaneously displaying the high magnification image of the segment at a position located by the marker on the low magnification image.

13. The method of Claim 5 including the step of shifting the marker to different segments and viewing at high magnification each of the selected segments; and marking on the low magnification image, each of the segments viewed at high magnification.

14. The method of Claim 5 including the steps of locating a computer-controlled microscope system used to make and store the digitized images on the Internet having a web site therefor; including the step of connecting an observer's computer and viewing screen over the Internet to the computer-controlled microscope at the web site in real time and transmitting the digitized signals for low magnification image and high magnification image from the web site to the observer's computer and viewing screen.

15. The method of Claim 5 including the step of enhancing the reconstructed, high magnification image by color filtering.

16. The method of Claim 5 including the step of providing a full color, low magnification, digitized image to the observer for aiding the observer in selecting a plurality of areas of interest on a priority basis.

17. The method of Claim 5 including the steps of:

analyzing the specimen on the slide with image analysis tests; and

22. The method of Claim 21 wherein the step of scanning and digitizing at low magnification further includes the steps of:

positioning and coordinating spatially a plurality of low magnification image tiles spatially to provide a composite macro image of the specimen formed from the plurality of low magnification image tiles; and

providing a macro image of the specimens having a field of view substantially larger than the field of view yielded by the objective lens used for the low magnification scanning.

23. A method of using digitized, microscope images of a specimen having images at a first resolution at a low magnification and images at a second resolution at a higher magnification, the method comprising the steps of:

showing to a user a series of digitized, tiled and stored images abutted to form a macro image from the specimen at a first resolution;

selecting from the stored macro image an area thereon to the viewer to be viewed at higher resolution;

showing to the user a series of abutted, digitized, tiled, stored images at the higher magnification and higher resolution to the viewer to be viewed at higher resolution.

24. A method in accordance with Claim 23 including the further step of shifting by user, back and forth between lower and higher resolution, stored images to analyze the specimen.

25. A method in accordance with Claim 24 including the further step of marking on the stored macro image the area to be viewed at higher resolution.

28. A method in accordance with Claim 27 including the step of marking on the macro image, the location of the micro image, so that the observer understands where the micro image is located on the macro image.

29. A method in accordance with Claim 27 including the step of selecting several segments each for viewing as a micro image and marking the location of each micro image on the composite image.

30. A method in accordance with Claim 29 including the step of recording the macro image with the marks thereon to provide a record of the segments viewed by the observer at high magnification to provide an audit trail for a later auditing of the segments viewed at higher resolution.

31. A method of acquiring and analyzing biological specimens with a computer-controlled microscope; said method comprising the steps of:

providing a biological specimen in position for scanning through the computer-controlled microscope;

scanning and digitizing the biological specimen through the microscope at a first, low magnification and acquiring and storing a digitized image of the specimen in color;

displaying the stored, low magnification, digitized image in color to an observer to provide a macro image for viewing by the observer;

selecting a segment of the colored, digitized, low magnification image for viewing at a higher magnification than the low magnification;

scanning the segment at the higher magnification and acquiring a colored digitized image of the segment at the higher magnification to provide a micro image of the segment;

an imaging subsystem connected to a microscope to acquire and digitize the images of the specimen;

a first optical system and image acquisition and image storing system having a first objective lens to acquire and arrange a stored series of images at a low magnification to provide a macro digitized image of the specimen having a field of view larger than the field of view from the objective lens used for the low magnification acquisition of images;

a second optical and image acquisition and image storing system having a second, higher magnification objective lens to acquire and arrange a stored series of images at higher magnification and to provide a micro view image of the segment having a field of view larger than the field of view from the higher magnification objective lens.

35. A system in accordance with Claim 34 wherein the first image subsystem includes an X and Y position storage device for storing the X and Y coordinates for the macro image.

36. A system in accordance with Claim 34 wherein the second image acquisition subsystem has a subsystem for reconstructing high magnification image tiles with their X and Y coordinates positioned to reproduce spatially high resolution image tiles into the micro image as if the micro image was in the original image.

37. A system in accordance with Claim 34 wherein the first image acquisition subsystem has a subsystem for reconstructing low resolution image tiles with their X and Y coordinates positioned to reproduce spatially the low resolution tiles into a macro image of the specimen.

40. A method for analyzing biological specimens by an image analysis system having a computer-controlled, automated microscope comprising the following steps:

placing a biological specimen in a microscope for viewing;

using a computer terminal to control the image analysis system and microscope to acquire low magnification, image tiles of the specimen and to provide low magnification, composite, macro image from the plurality of image tiles;

displaying the macro image on a screen of the computer terminal;

interactively selecting at least one point of interest on the displayed macro, specimen image for viewing at a higher magnification;

sending signals from the computer terminal to control the image analysis system and the computer-controlled microscope to acquire a plurality of higher magnification, image tiles and to provide a low magnification, composite micro image; and

analyzing the micro images of the specimen.

41. A storage medium having digitized images of a specimen from a microscope slide comprising:

a storage medium;

a first series of contiguous, multiple digitized images at a first magnification abutted against each other to create an overall low resolution view of several adjacent, original microscope images assembled together;

the first series of multiple, digitized images taken from at least a portion of a specimen on the slide;

a second series of contiguous, multiple, digitized images at a second higher magnification abutted against each other to create a high resolution view of several adjacent, original microscope images assembled together, taken from said portion of the specimen; and

Fig. 1

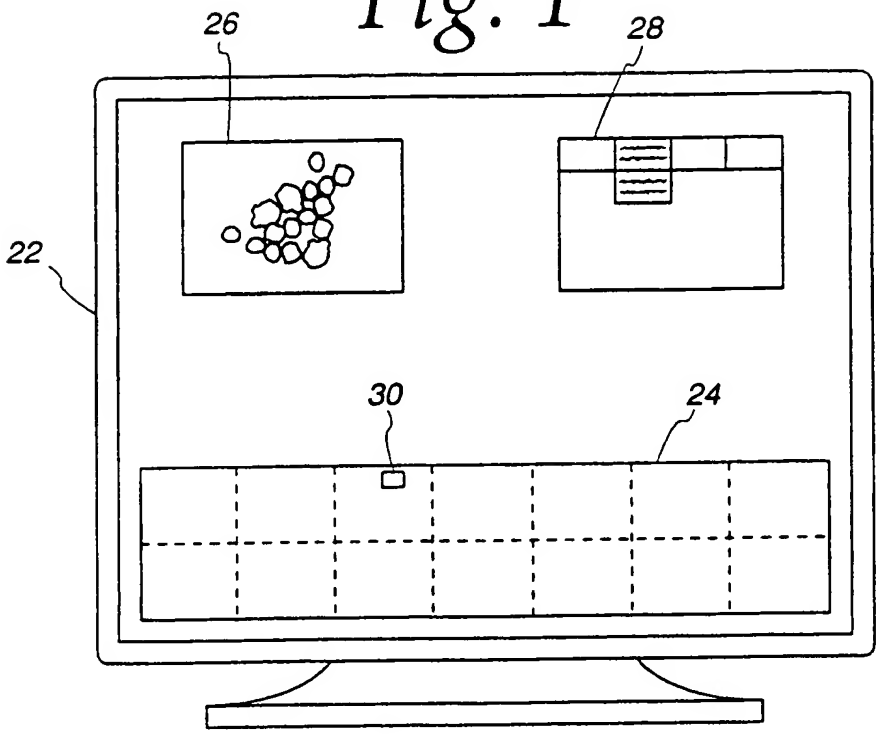
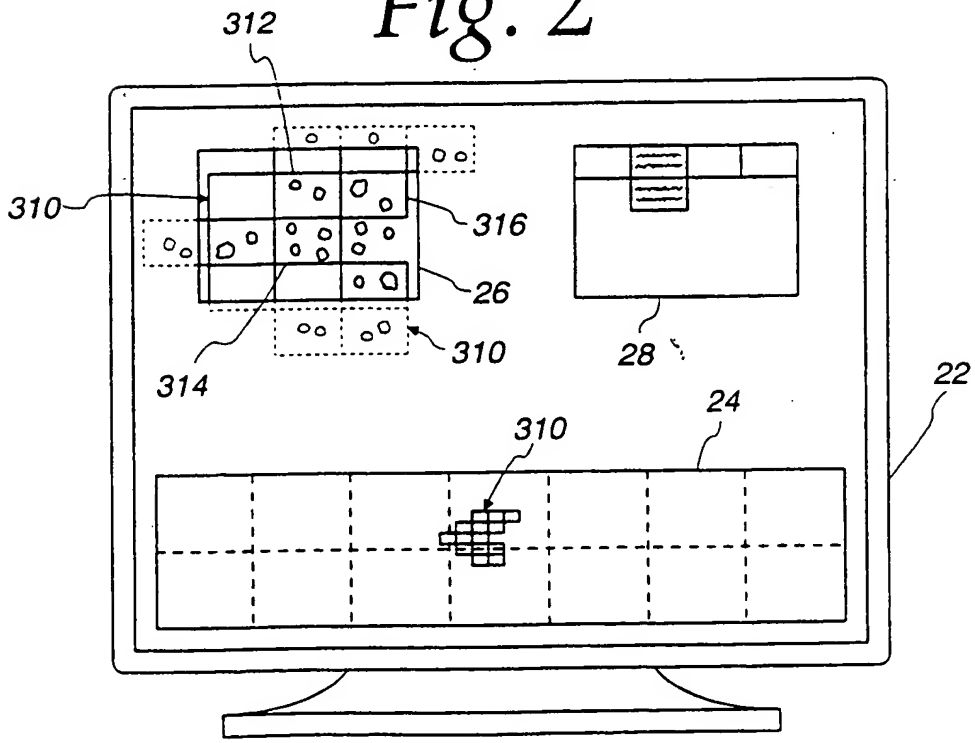


Fig. 2



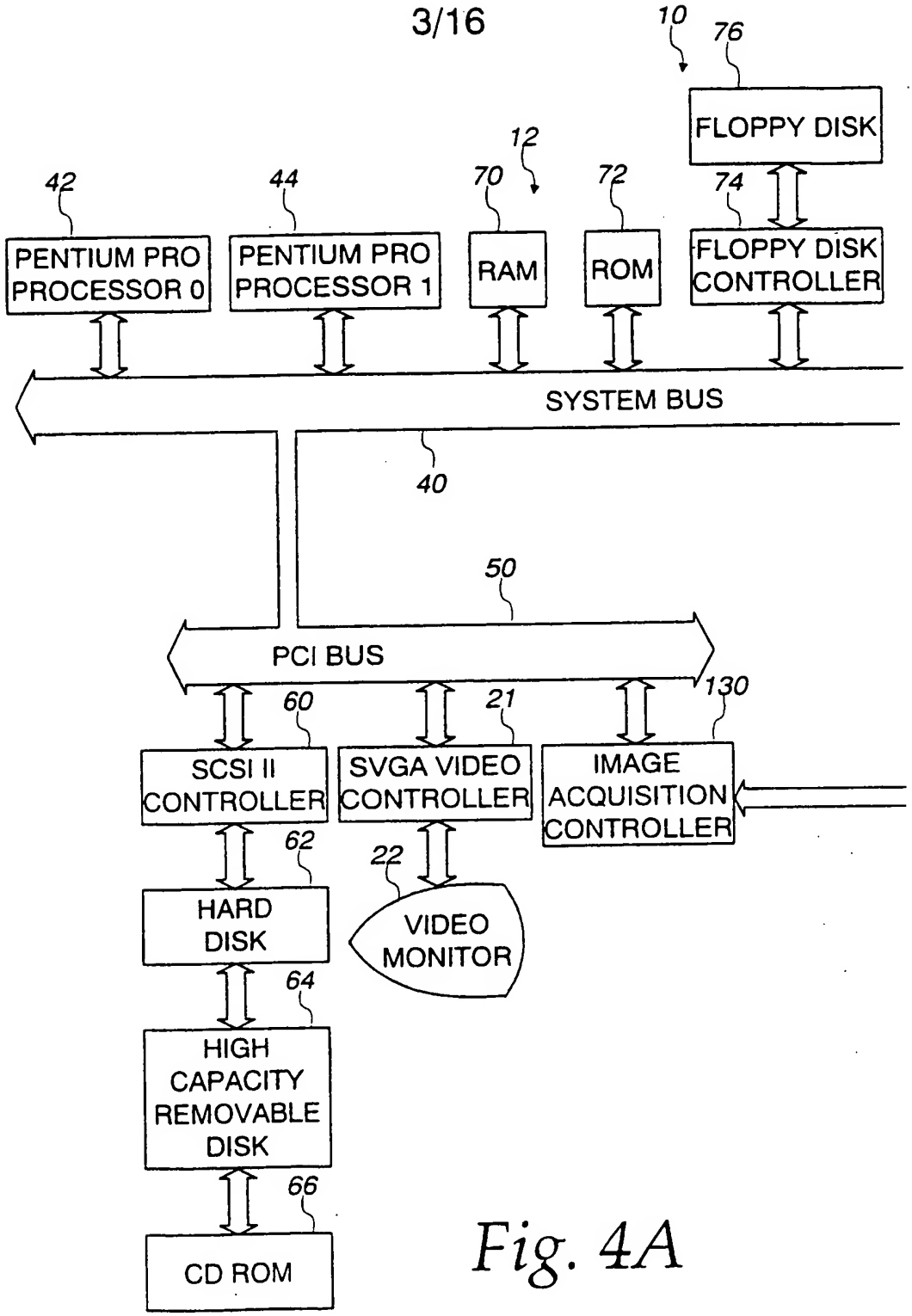


Fig. 4A

Fig. 5

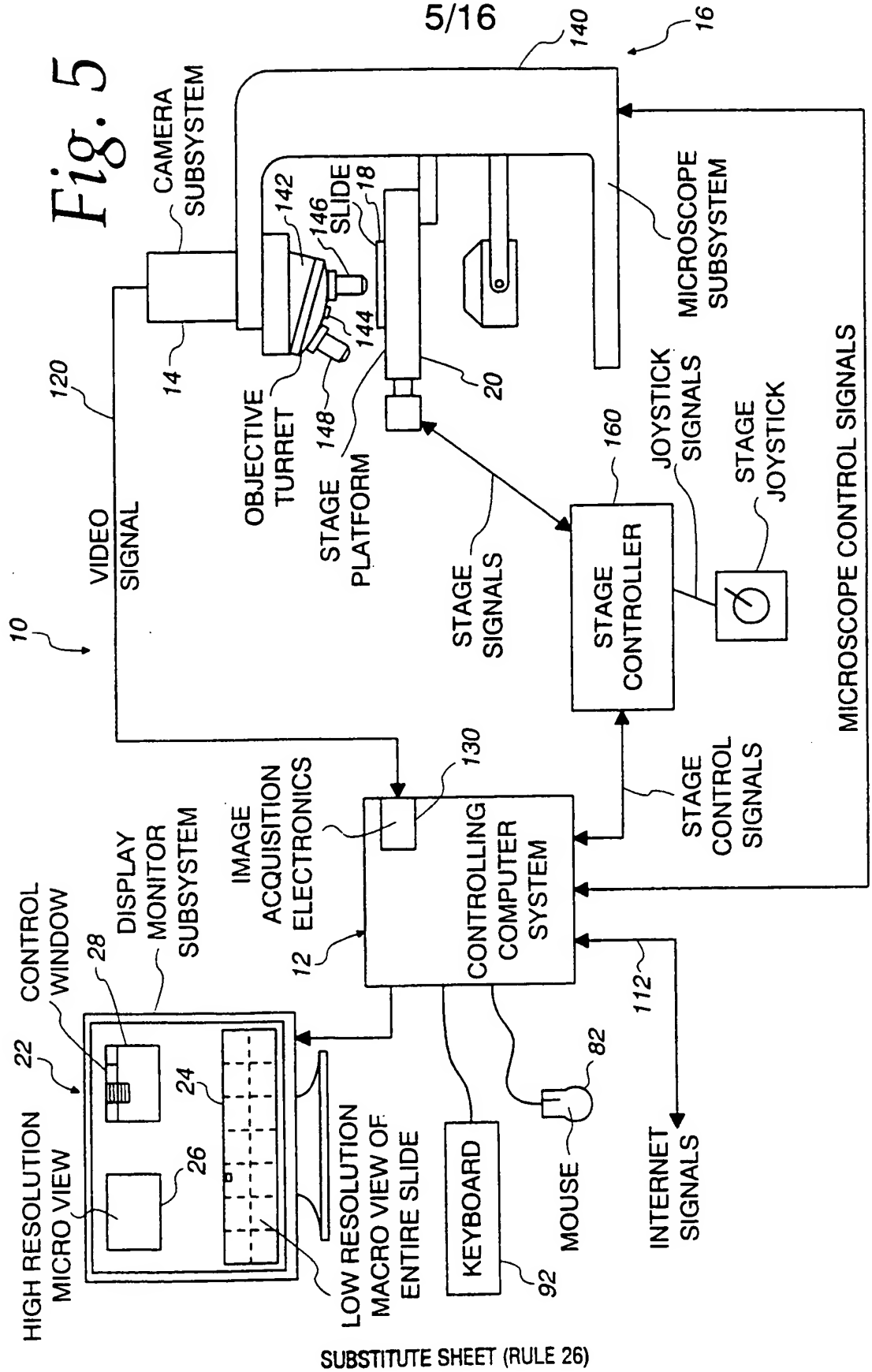


Fig. 7

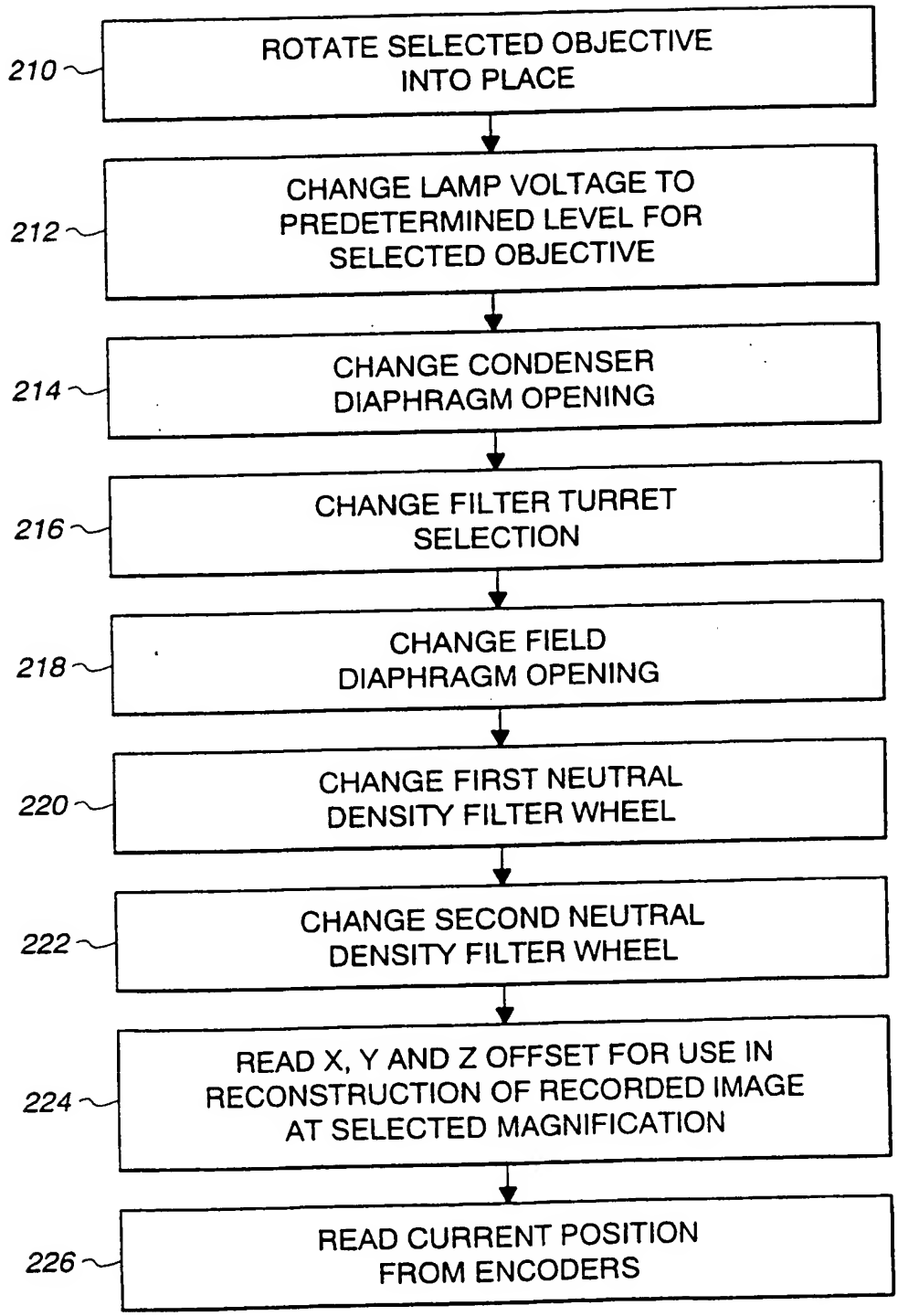

210
ROTATE SELECTED OBJECTIVE INTO PLACE
212
CHANGE LAMP VOLTAGE TO PREDETERMINED LEVEL FOR SELECTED OBJECTIVE
214
CHANGE CONDENSER DIAPHRAGM OPENING
216
CHANGE FILTER TURRET SELECTION
218
CHANGE FIELD DIAPHRAGM OPENING
220
CHANGE FIRST NEUTRAL DENSITY FILTER WHEEL
222
CHANGE SECOND NEUTRAL DENSITY FILTER WHEEL
224
READ X, Y AND Z OFFSET FOR USE IN RECONSTRUCTION OF RECORDED IMAGE AT SELECTED MAGNIFICATION
226
READ CURRENT POSITION FROM ENCODERS

Fig. 9

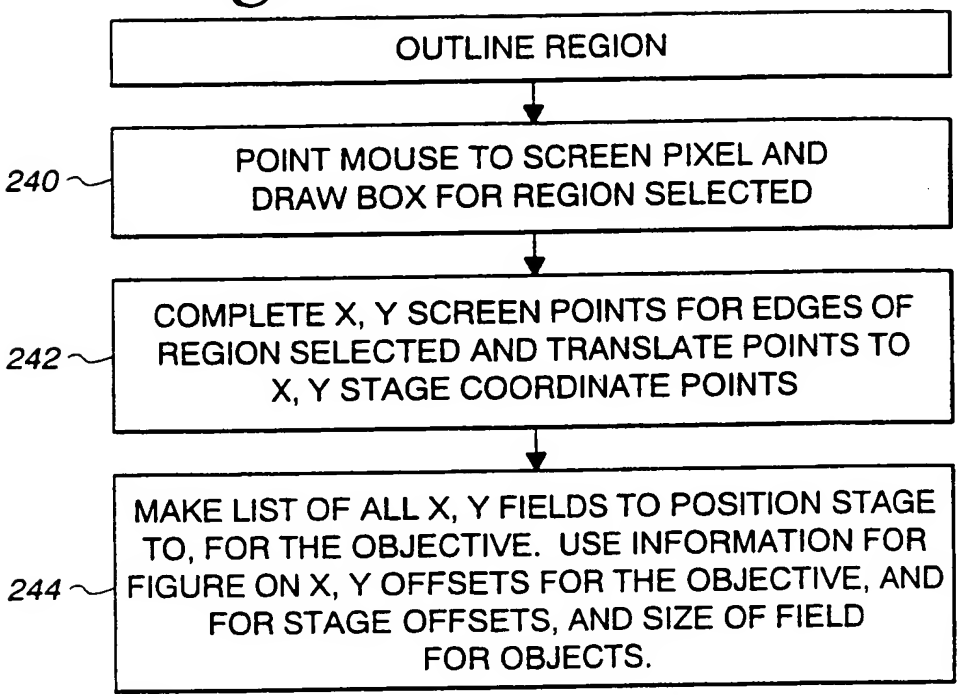


Fig. 10

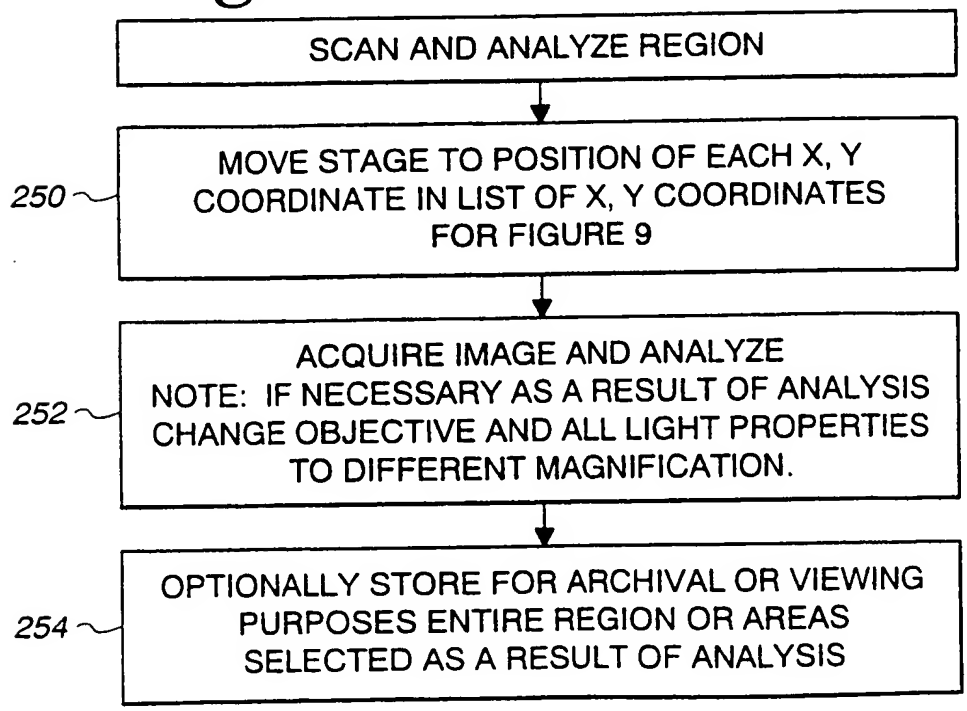


Fig. 12

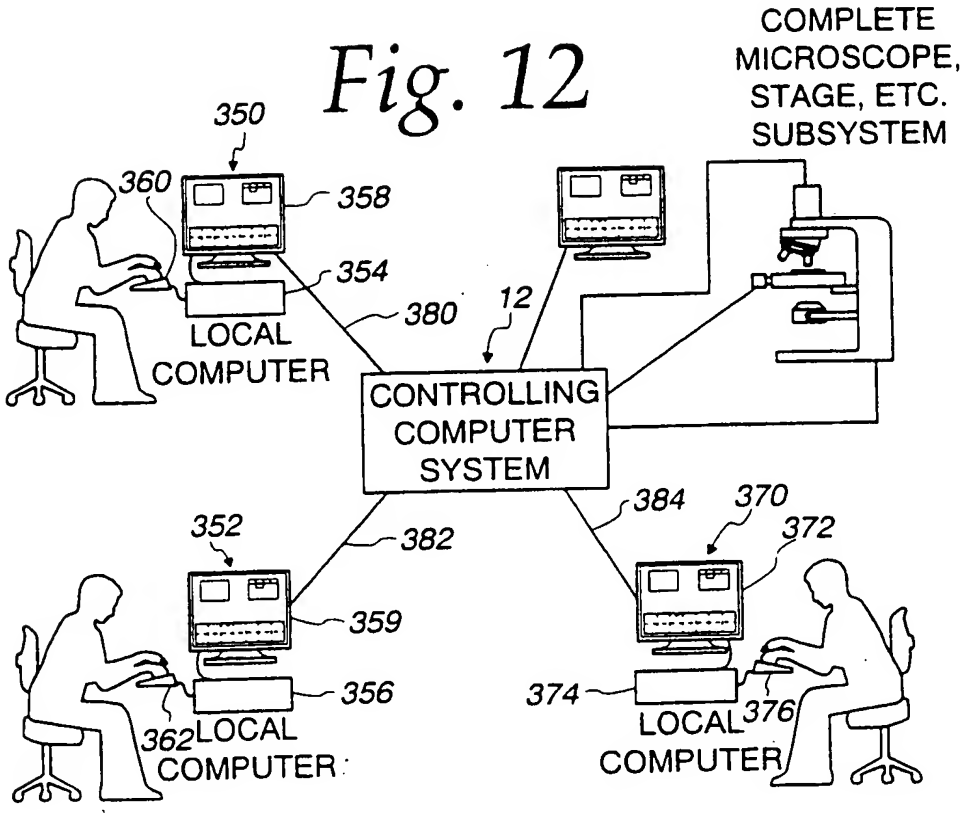


Fig. 12A

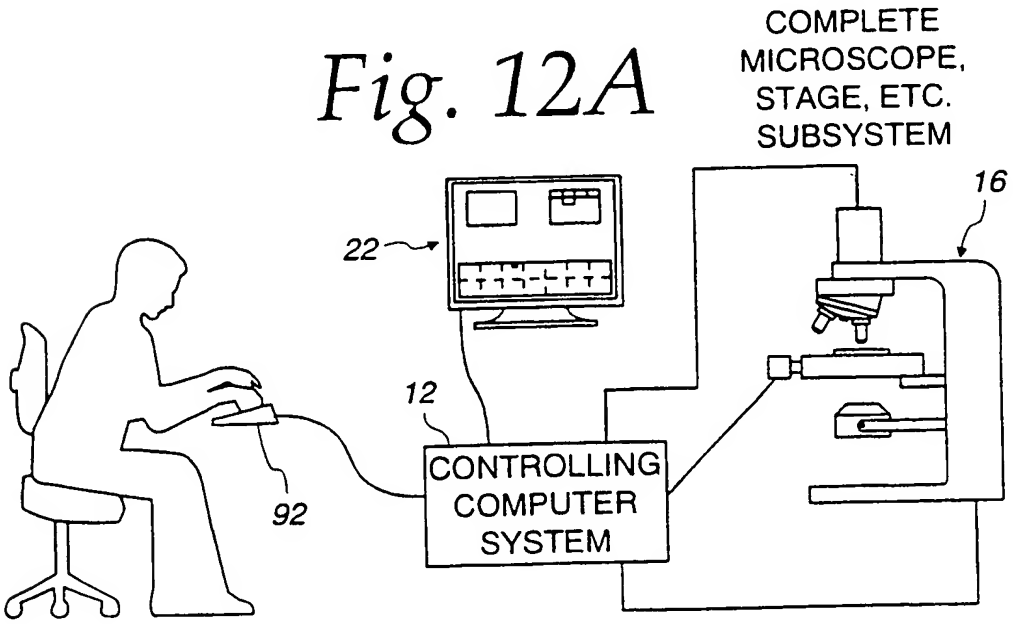


Fig. 14

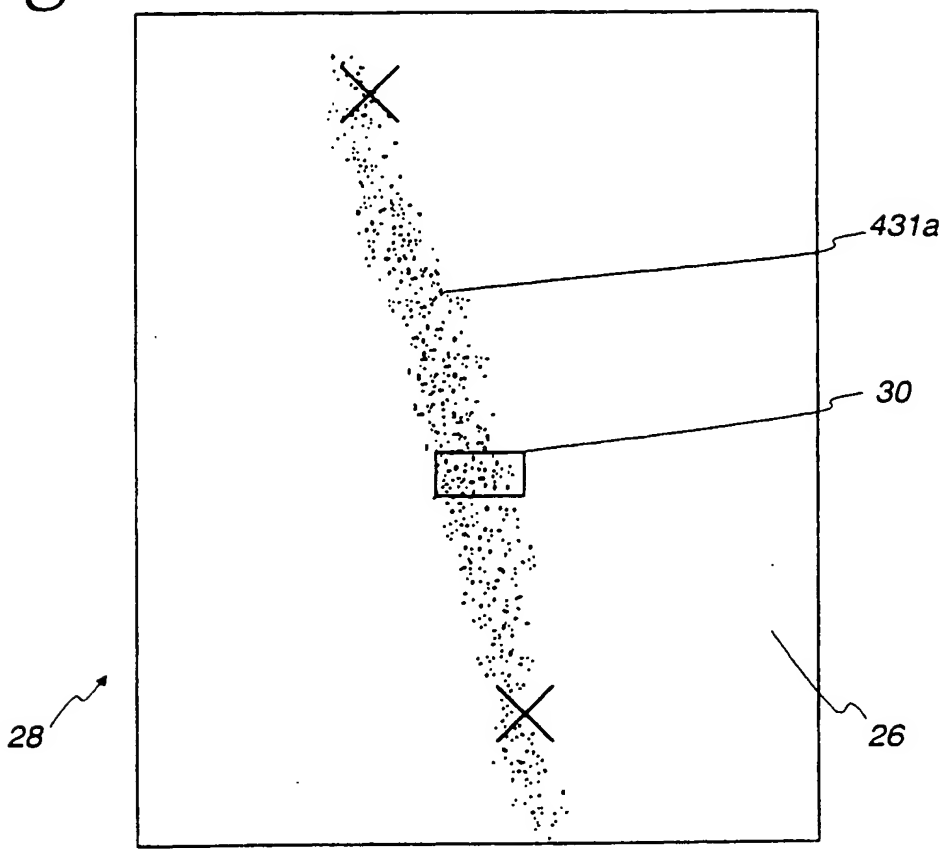


Fig. 14a

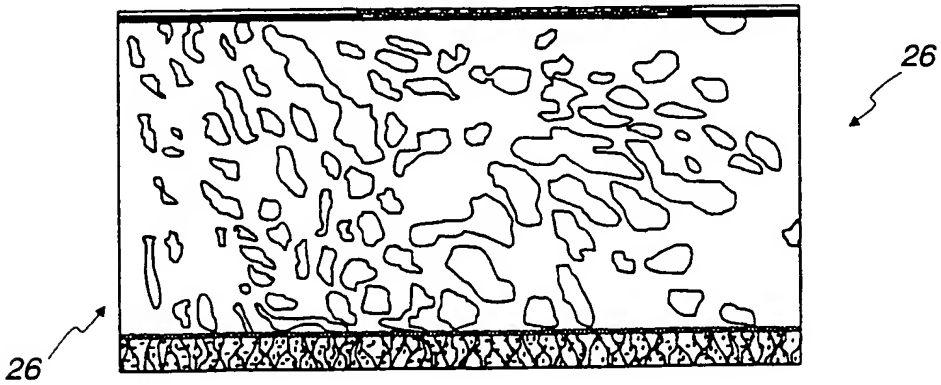


Fig. 16

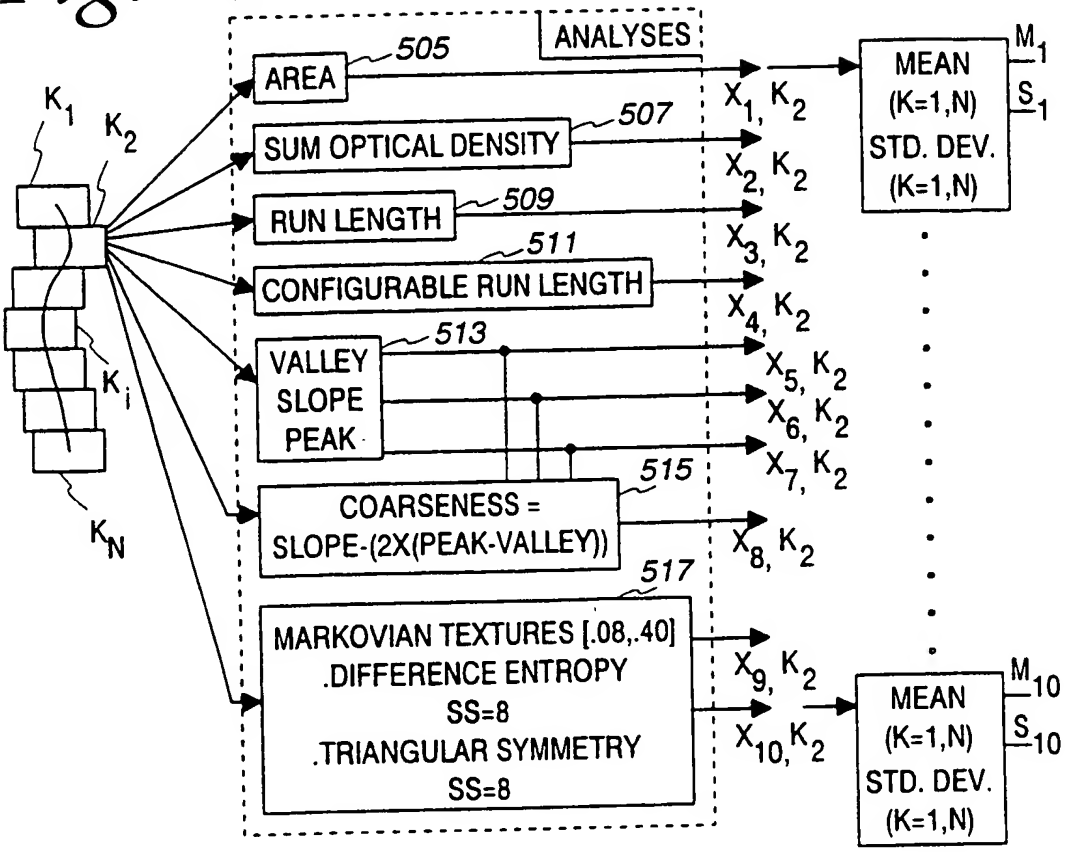
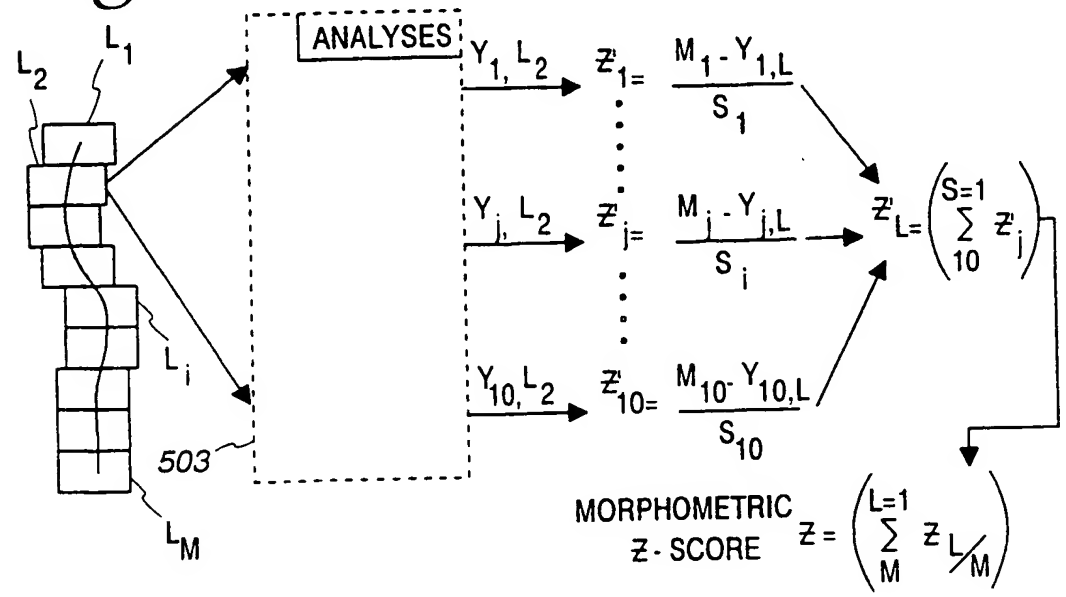


Fig. 16A (SUSPECT TISSUE)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/04208

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G06K 9/00
US CL :382/133

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 382/128, 133, 134, 284, 294, 318, 319; 128/922

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,257,182 A (LUCK et al) 26 October 1993, Figures 1 & 2; column 3, line 23 through column 5, line 12; column 7, line 1 through column 8, line 5.	1-46
Y	US 4,287,272 A (RUTENBERG et al) 15 February 1994, Figure 3A; column 4, lines 39-62; column 12, lines 19-56; column 13, lines 27-61.	1-46
A	US 5,544,650 A (BOON et al) 13 August 1996, Figure 2; column 4, lines 32-65.	1-46
A	US 5,428,690 A (BACUS et al) 27 June 1995, column 3, lines 12-38.	1-46
A	US 5,252,487 A (BACUS et al) 12 October 1993, see the entire document.	1-46

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 JUNE 1998	Date of mailing of the international search report 09 SEP 1998
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Form PCT/ISA/210 (second sheet)(July 1992)*